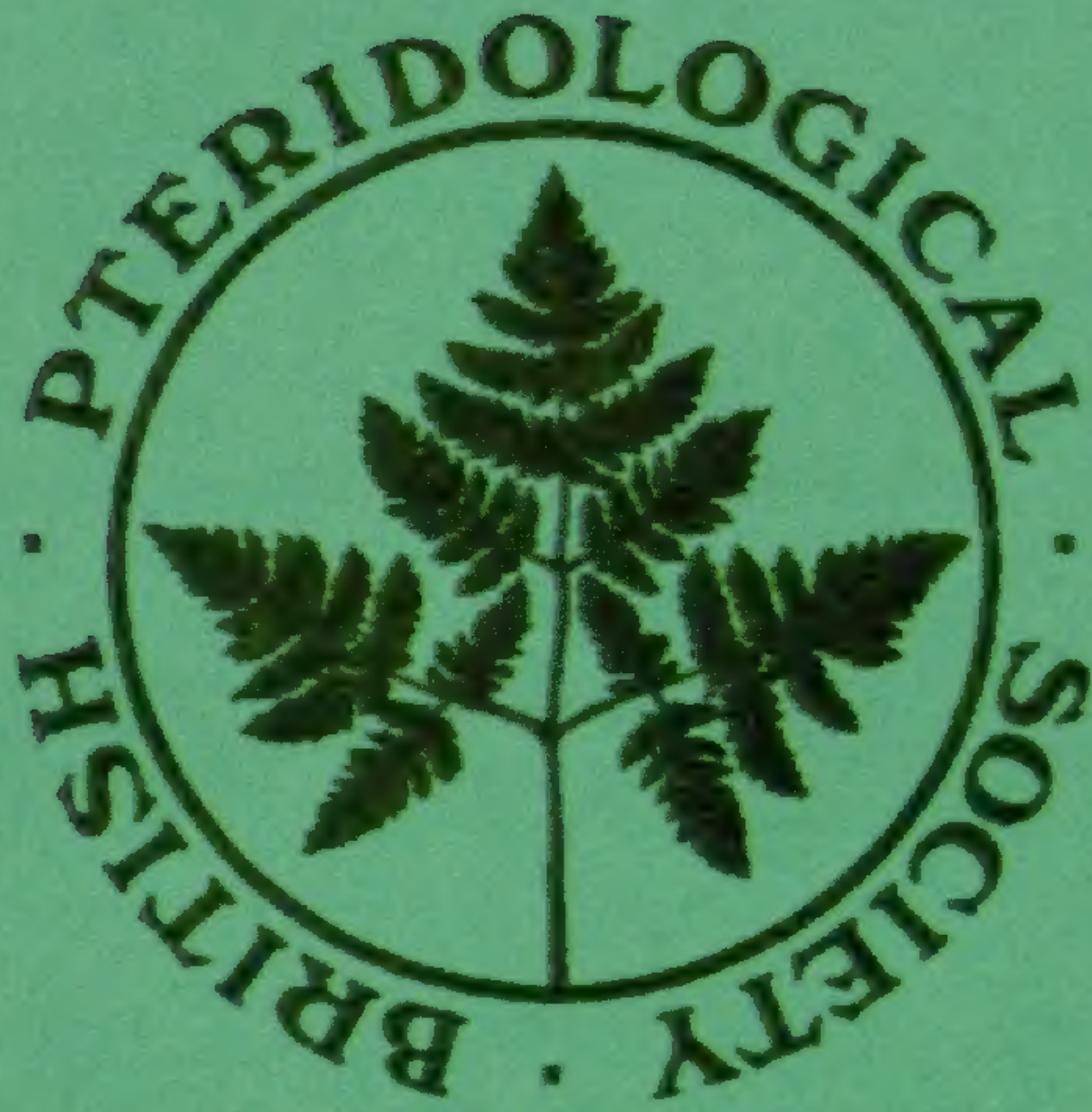




**THE
FERN
GAZETTE**

EDITORS:
M. Gibby & A. Leonard

THE FERN GAZETTE is a journal of the British Pteridological Society and contains peer-reviewed papers on all aspects of pteridology.

Manuscripts may be submitted, and books etc. sent for review, to: Prof. M. Gibby,
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THE FERN GAZETTE Volume 18 Part 1 was published on 5th May 2007

Published by THE BRITISH PTERIDOLOGICAL SOCIETY
c/o Department of Botany,
The Natural History Museum, Cromwell Road, London SW7 5BD, UK

Printed by Bishops Printers Limited
Fitzherbert Road, Farlington, Portsmouth, PO6 1RU, UK
www.bishops.co.uk

Cover design by Hazel Sims

TWO NEW SPECIES OF *SELAGINELLA* SUBGENUS *HETEROSTACHYS* (SELAGINELLACEAE) FROM THE GUIANAS

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Key words: *Selaginella*, *Heterostachys*, Guyana, Suriname, French Guiana, South America.

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ABSTRACT

During our study of the biodiversity of the Guianas and the preparation of the flora of this region, we discovered that the I. Valdespino's monograph of the genus *Selaginella* subgen. *Heterostachys* was never published. Two taxa, *S. gynostachya* and *S. karowtipuensis*, presented in this monograph and new to the Guianas, are published herein.

GARDEN LIBRARY

Mots clés: *Selaginella*, *Heterostachys*, Guyana, Surinam, Guyane, Amérique du Sud.

RÉSUMÉ

Dans le cadre de notre étude de la biodiversité des Guyanes et de la rédaction de la flore de cette région, nous avons constaté que la monographie de I. Valdespino sur le genre *Selaginella* subgen. *Heterostachys* n'a jamais été publiée. Deux taxons, *S. gynostachya* et *S. karowtipuensis*, présentés dans cette monographie et nouveaux pour les Guyanes, sont publiés ici.

INTRODUCTION

In 1995 Ivan A. Valdespino Quintero completed a monographic study to obtain his Doctor of Philosophy degree in Botany from the Graduate School of The City University of New York. This work was supervised by J.T. Mickel as Chair of the Examining Committee. Certain elements of the dissertation, such as the lectotypifications of *S. flagellata* Spring and of *S. minima* Spring, are cited by Mickel & Smith (2004), but unfortunately Valdespino's monograph was never entirely published.

During our study of the biodiversity of the flora of French Guiana and in relation to the international programme of the 'Flora of the Guianas', we had to consult this work. For instance, *S. minima*, of which the type is from French Guiana, is treated in this work. This species was known only from a few specimens collected before 1850 but has been recently found again in French Guiana (Cremers & Boudrie, 2006). We are also preparing a paper dealing with 'Pteridophytes having at least a syntype collected in the Guianas' and fascicle 9 of the Pteridophytes of the *Flora of the Guianas*, both works which enhance the interest of such a monograph in which two new species have not been officially published.

We present here the descriptions of these two new species to the Guianas, with type specimens from Guyana. What follows has been extracted verbatim from Valdespino's

monograph. Valdespino, who is currently unavailable, deserves full credit for recognizing and distinguishing these two new species. Here we intend to validate and to pay tribute to his work.

NEW SPECIES

Selaginella gynostachya Valdespino ex Cremers & Boudrie, **sp. nov.** (Figure 1 a, b, c; Figure 2 a, b, c)

A *S. hyalogramma* Valdespino et *S. karowtipuensis* Valdespino foliis intermediis basi subtruncatis vel rotundatis nec non foliis lateralibus supra idioblastis destitutis diversa; ulterius a prima foliis intermediis ellipticis, ovato-ellipticis vel ovatis, foliis lateralibus oblongis vel oblongo-ovatis necnon megasporis pallide luteis; ulterius a secunda foliis intermediis apice acutis vel breviter acuminatis.

Type: GUYANA. Upper Potaro River, vicinity of Kopinang village, 4°58'N, 59°50'W, 670 m, 29 Jul. 1989, *Boom & Samuels* 8939 (holotype NY; isotype BRG).

Etymology –Gr., gyne, female or pertaining to female organs, and stachys, spike; referring to the mostly megasporangiate strobili found on specimens of this species.

Plants terrestrial. Stems creeping, stramineous, 5-14 cm long, 0.4-0.8 mm diam., not articulate, not flagelliform, not stoloniferous, 1- or 2-branched. Rhizophores axillary, restricted to basal ½ of the stem, stout, (0.28-) 0.34-0.56 mm diam. Leaves dimorphic throughout, membranaceous, the upper surface green, composed of elongate cells with sinuate walls. Lateral leaves distant, slightly ascending, oblong to oblong-ovate, 2.8-4 x 1-1.9 mm; base subtruncate to rounded, the acroscopic base strongly overlapping the stem, the basisopic base not overlapping the stem; margins slightly hyaline, or pale green, the acroscopic margin serrate to ciliate-denticulate along basal 1/3, serrulate apically, the basisopic margin entire to serrulate apically; apex obtuse to broadly acute, dentate at tip; both surfaces glabrous, the upper surface without idioblasts, the lower surface frequently with conspicuous or obscure idioblasts, the stomata present only on the lower surface. Median leaves distant, ascending, elliptic to ovate-elliptic, 1.7-2.3 x 0.7-1.4 mm; base asymmetric with the inner base truncate and the outer base with a slightly developed auricle; margins slightly hyaline, serrate to serrulate; apex acute to shortly acuminate, the acumen, when present, less than 1/3 the length of the lamina, 0.1-0.26 mm long, puberulent and dentate at tip; both surfaces glabrous, the upper surface frequently with conspicuous idioblats and stomata, the lower surface with obscure to conspicuous idioblasts, and without stomata. Axillary leaves similar to lateral leaves or more broadly ovate, except the base frequently rounded. Strobili terminal on branch tips and main stem apex, lax, flattened, and dorsiventral, 5-15 mm long. Sporophylls dimorphic, basally attached to the axis; dorsal sporophylls green, spreading, basally attached to the axis, with an adaxial flap extending along ca. ½ to ¾ the sporophyll length, asymmetric or lanceolate to lance-ovate, 1.6-2.36 x 0.6-0.98 mm, with a strongly developed and slightly denticulate keel along midrib, the base ± rounded, the margins slightly hyaline to hyaline greenish, serrate to shortly ciliate, the apex acute to shortly acuminate, the upper surface green, composed of ± rounded cells, except for the portion that folds to form the flap where the cells hyaline and elongate with sinuate walls, glabrous, frequently with conspicuous idioblasts and stomata, the



Figure 1a. Scanned photograph of *Selaginella gynostachya* Valdespino ex Cremers & Boudrie (Cremers 5379, CAY): Herbarium sheet.

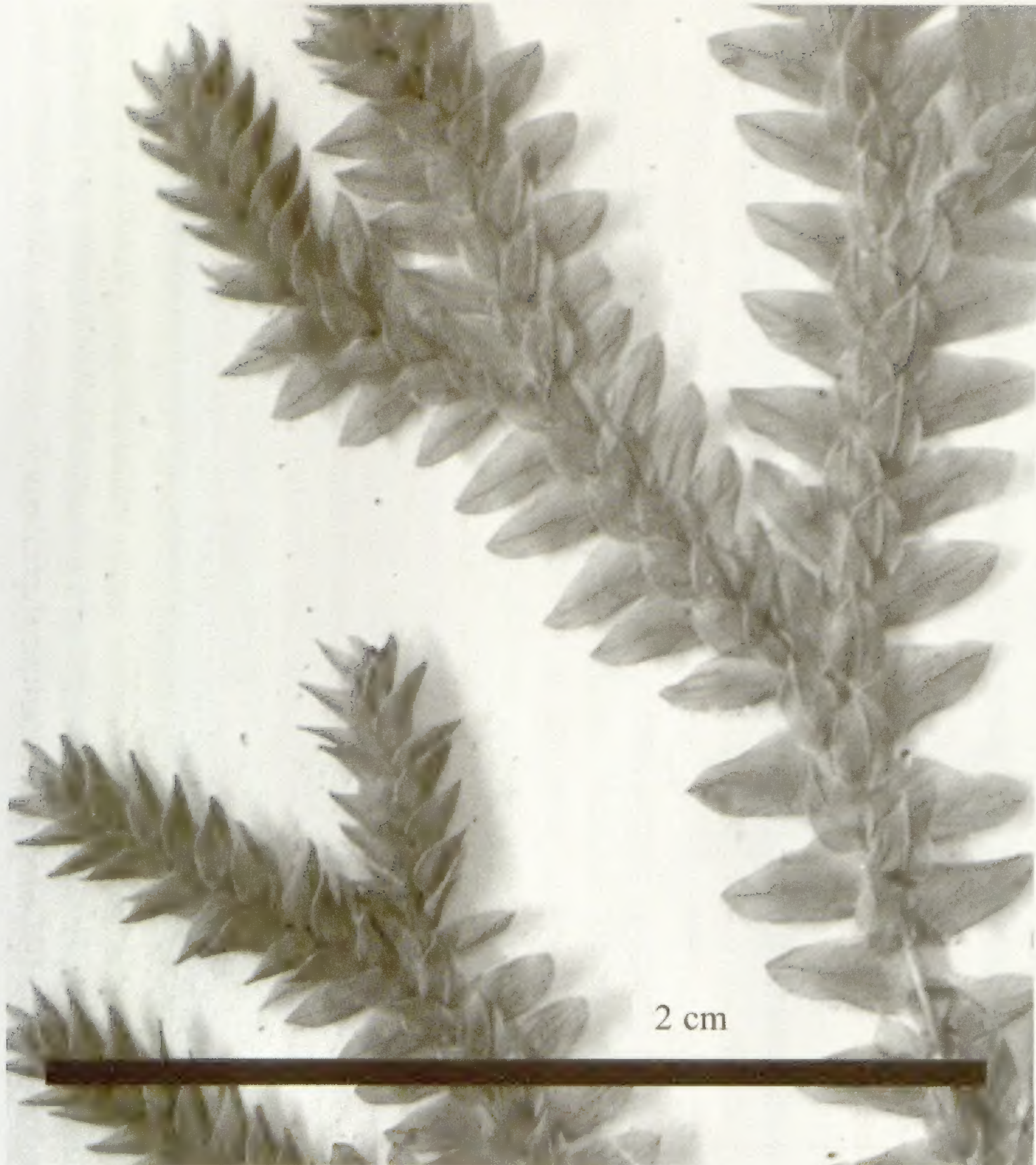


Figure 1b. Scanned photograph of *Selaginella gynostachya*. Detail of the adaxial face.



Figure 1c. Scanned photograph of *Selaginella gynostachya*. Detail of the abaxial face.

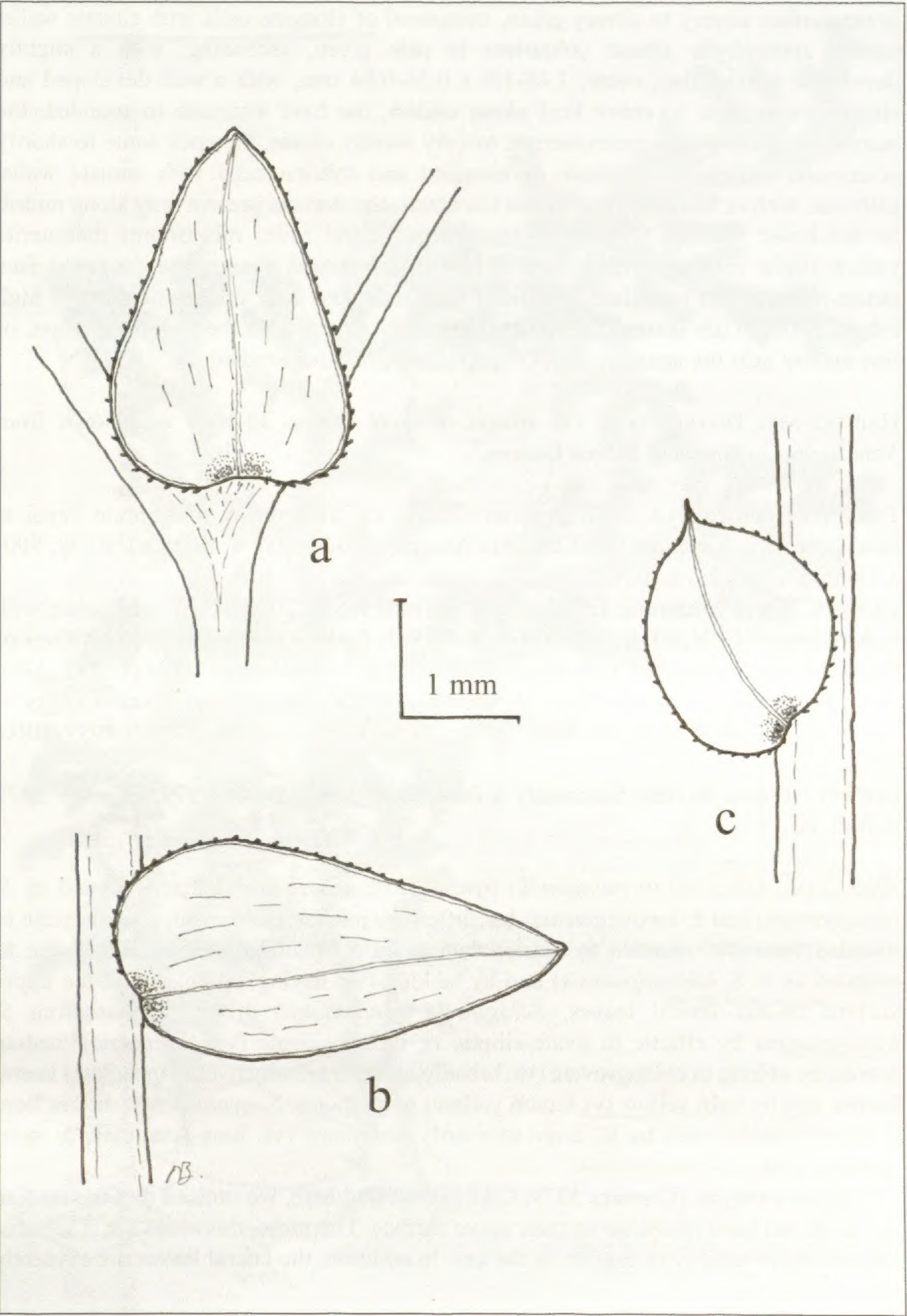


Figure 2. Drawings of the leaves of *Selaginella gynostachya* Valdespino ex Cremers & Boudrie (Cremers 5379, CAY).
a = axillary leaf (abaxial face); b = lateral leaf (abaxial face); c = median leaf (adaxial face);
The small lines on the leaves represent the idioblasts (M. Boudrie's drawings).

lower surface silvery to silvery green, composed of elongate cells with sinuate walls; ventral sporophylls almost colourless to pale green, ascending, with a slightly developed adaxial flap, ovate, 1.48-1.9 x 0.56-0.84 mm, with a well developed and slightly denticulate to entire keel along midrib, the base attenuate to rounded, the margins hyaline to pale green, serrate to very shortly ciliate, the apex acute to shortly acuminate, both surfaces made of elongate and hyaline cells with sinuate walls, glabrous, with or without conspicuous idioblasts, the stomata present only along midrib on the lower surface. Megasporangia in two ventral rows; megaspores marguerite yellow (light yellow), with a well developed equatorial flange, the proximal face striate-reticulate to reticulate, the distal face reticulate with open reticulum of high ridges, 300-320 µm diam. Microsporangia mostly absent from the two dorsal rows, or few mature near the apex on dorsal rows; microspores not studied.

HABITAT AND DISTRIBUTION: On stream or river banks; 30-1000 m; known from Venezuela, Guyana, and French Guiana.

PARATYPES: VENEZUELA. Bolivar: Gran Sabana, ca. 10 km SW of Karauin Tepui at junction of Rio Karaurin Tepui and Rio Asadon (Rio Sanpa), 5°19'N, 61°03'W, 900-1000 m, *Liesner 23798* (UC).

GUYANA. Upper Mazaruni: East bank of Waruma River, 20 km S of confluence with Kako River, 5°19'N, 60°46'W, 1000 m, Feb 1985, *Renz 14188* (U); Essequibo River of Porto Bartica, *Jenman s.n.* (K); Macouria Riv., Nov 1886, *Jenman 2324* (BM, K, US); Macouria Creek, Essequibo River, *Jenman s.n.* (NY); Upper Potaro River: vicinity of Kopinang village, 4°58'N, 59°50'W, 670 m, 29 Jul 1989, *Boom & Samuels 8939* (BRG, NY).

FRENCH GUIANA. Rivière Sinnamary à Petit Saut, 30m, 12 Feb 1979, *Cremers 5379* (CAY!, Z).

TAXONOMIC DISCUSSION: *Selaginella gynostachya* seems most closely related to *S. hyalogramma* and *S. karowtipuensis* but differs by median leaves with a subtruncate to rounded base (vs. rounded to semicordate as in *S. hyalogramma* or subcordate to rounded as in *S. karowtipuensis*) and by lacking (vs. having) idioblasts on the upper surface of the lateral leaves. *Selaginella gynostachya* differs further from *S. hyalogramma* by elliptic to ovate-elliptic or broadly ovate (vs. lanceolate) median leaves, by oblong to oblong-ovate (vs. broadly ovate to broadly ovate-lanceolate) lateral leaves, and by light yellow (vs. lemon yellow) megaspores. *S. gynostachya* differs from *S. karowtipuensis* also by its acute to shortly acuminate (vs. long-acuminate to short aristate) apex.

On the paratype (*Cremers 5379*, CAY) illustrated here, we noticed that the median leaves do not have idioblasts on their upper surface. Therefore, this character is variable and should be used with caution in the key. In addition, the lateral leaves are evidently cilio-denticulate.

Selaginella karowtipuensis Valdespino ex Cremers & Boudrie, **sp. nov.** (Figure 3 a, b, c; Fig. 4 a, b, c).

Selaginella seemannii Baker affinis, sed foliis lateralibus, intermediis et sporophyllis superne idioblastis provisis diversa. A *S. stenophylla* A. Braun foliis intermediis basi

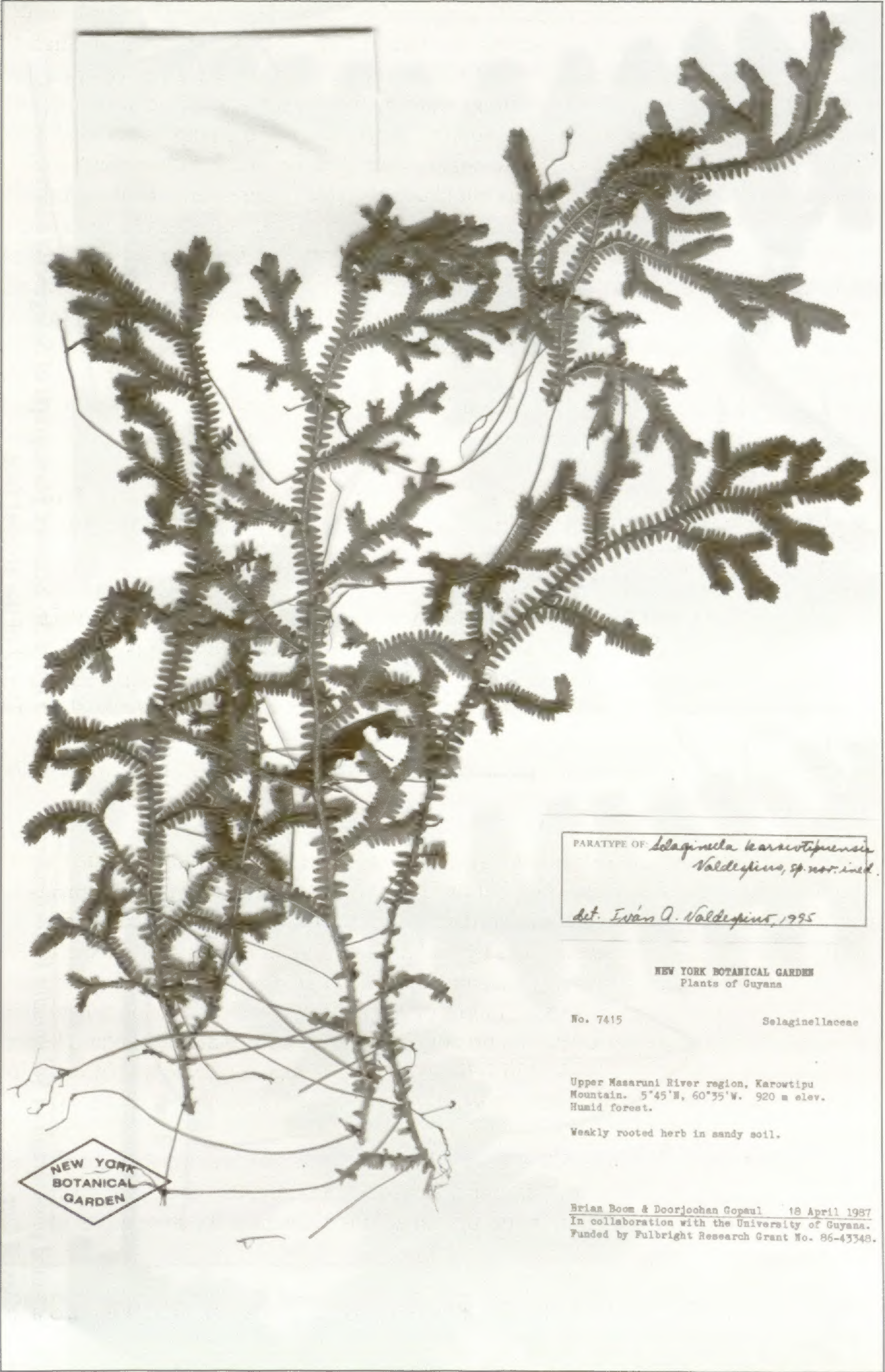


Figure 3a. Scanned photograph of *Selaginella karowtipuensis* Valdespino ex Cremers & Boudrie (Boom & Gopaul 7415, NY): Herbarium sheet.

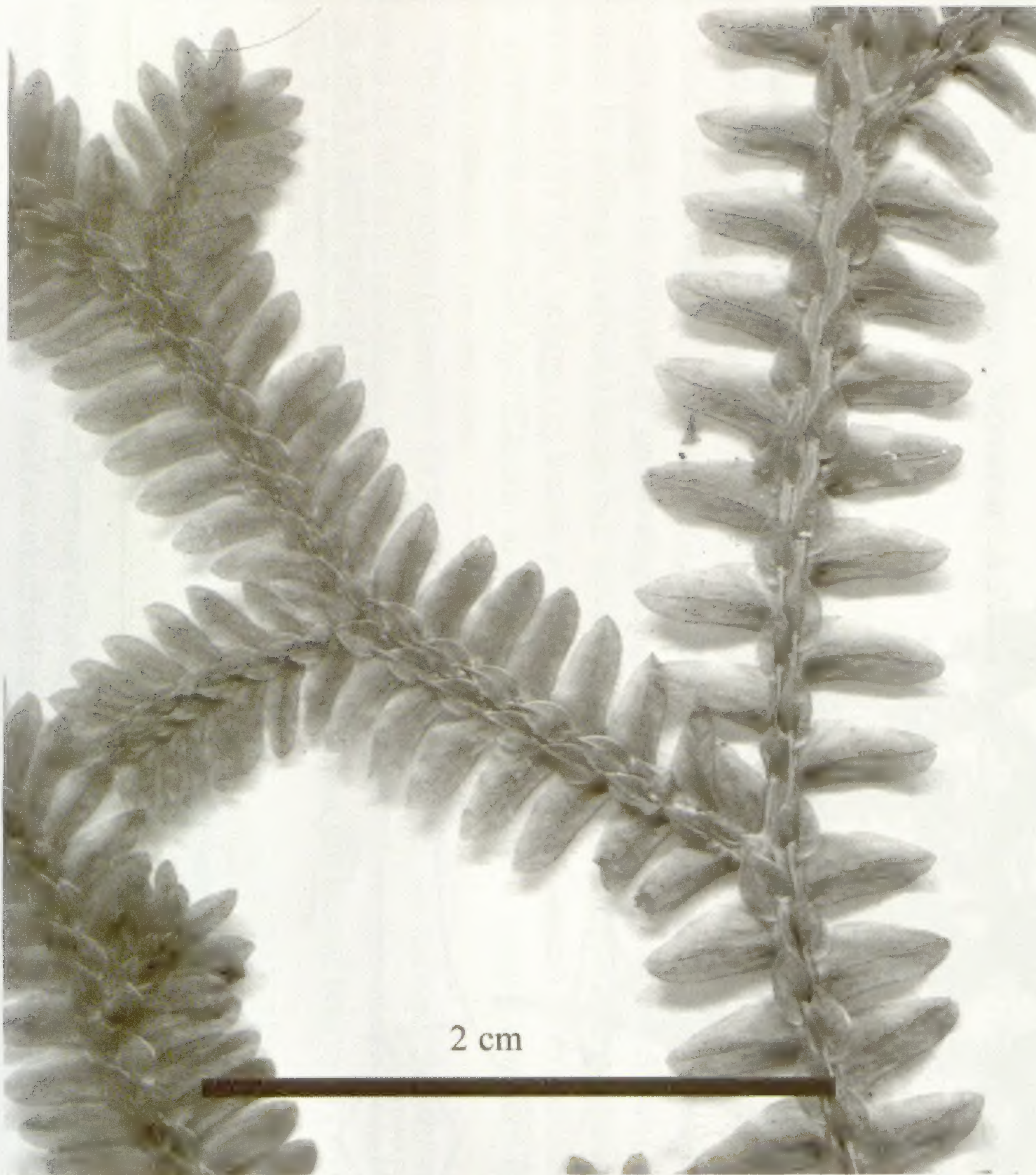


Figure 3b. Scanned photograph of *Selaginella karowtipuensis*. Detail of the adaxial face.

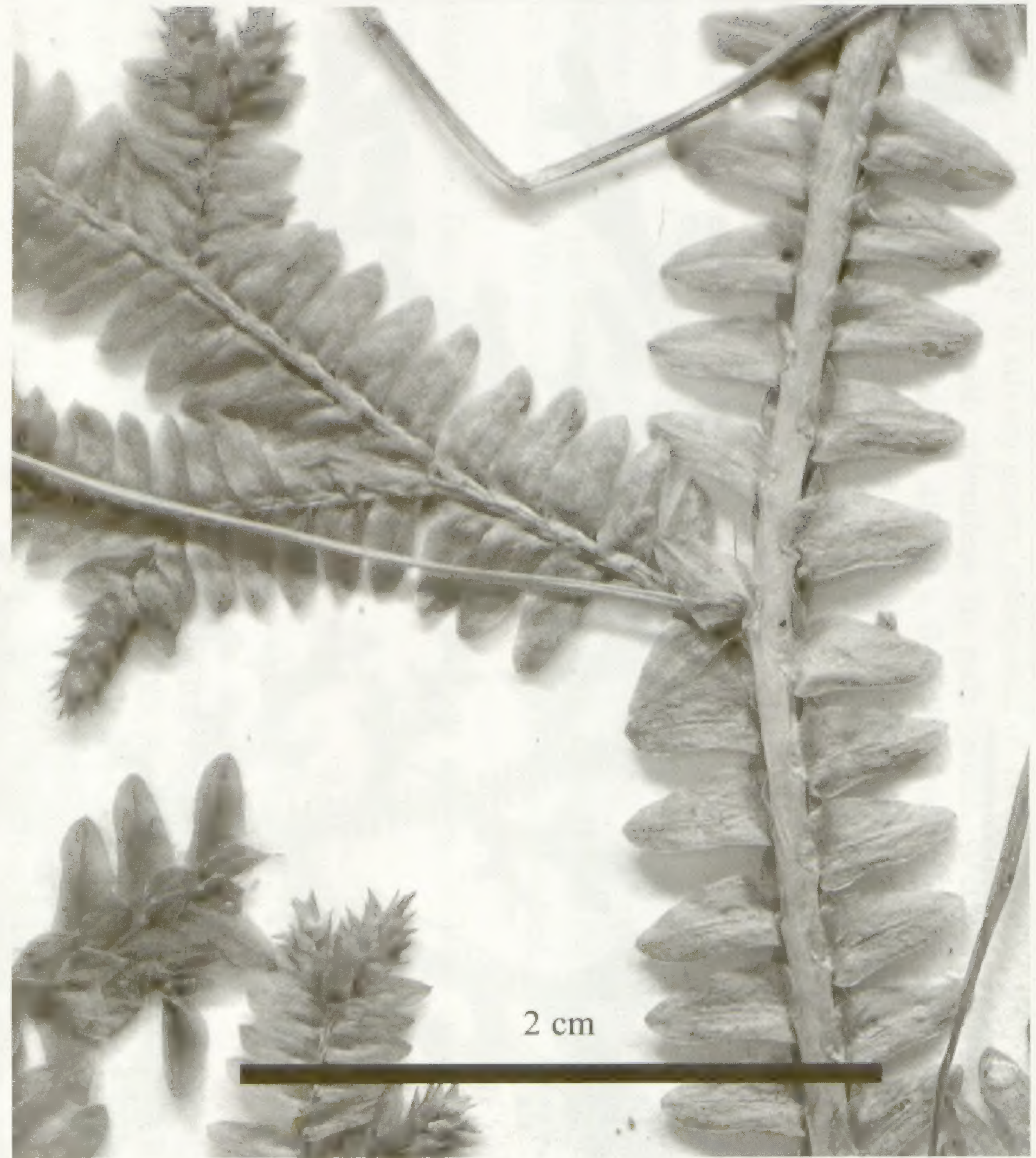


Figure 3c. Scanned photograph of *Selaginella karowtipuensis*. Detail of the abaxial face.

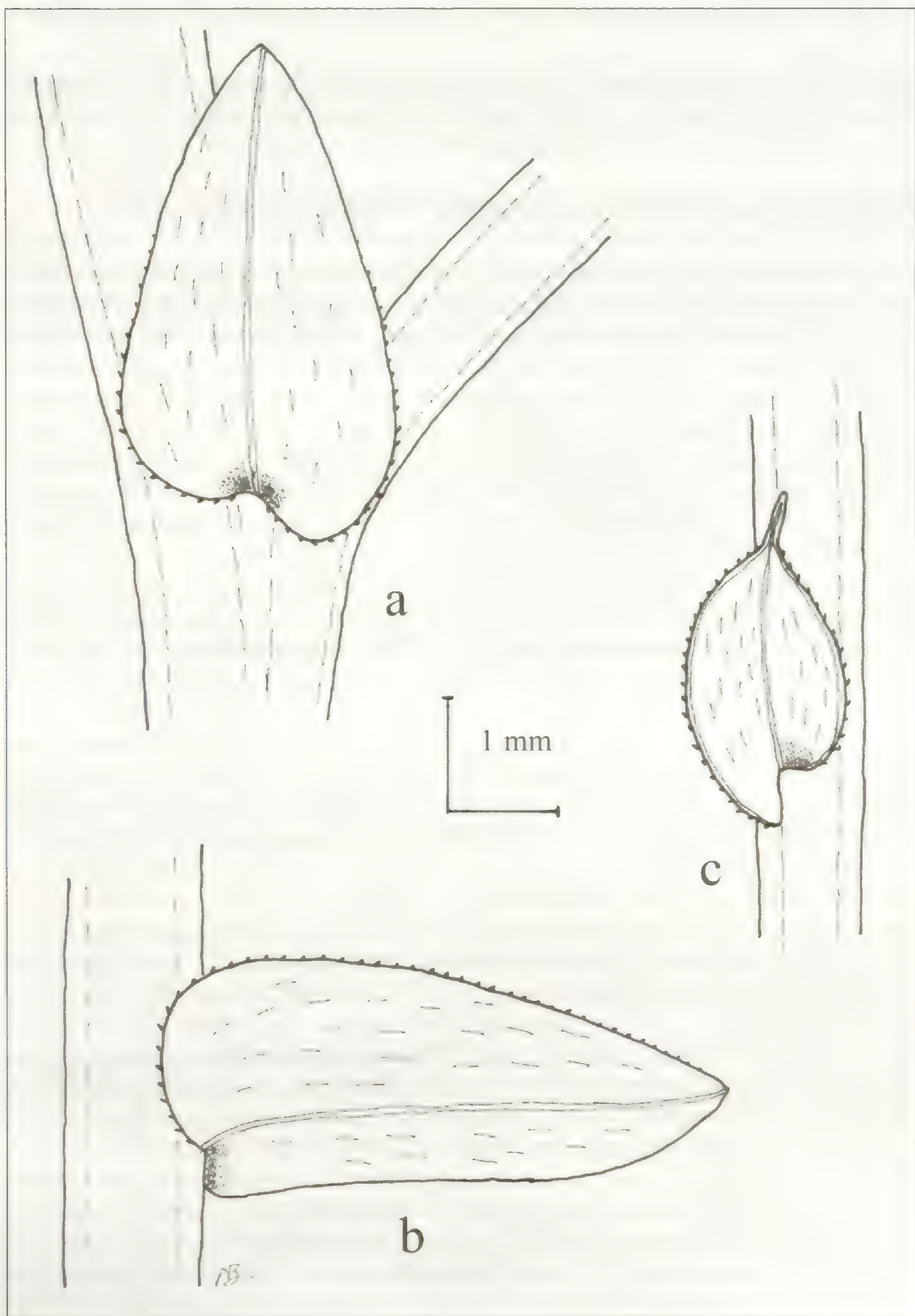


Figure 4. Drawings of the leaves of *Selaginella karowtipuensis* Valdespino ex Cremers & Boudrie (*Boom & Gopaul 7415*, NY).

a = axillary leaf (abaxial face); b = lateral leaf (abaxial face); c = median leaf (adaxial face);

the small lines on the leaves represent the idioblasts (M. Boudrie's drawings).

non nisi auriculo exteriori vel serrulato provisus diversa.

Type: GUYANA. Upper Mazaruni: Karowtipu Mountain, between camp and peak on western side of mountain, 5°45'N, 60°35'W, 920-1080 m, 24 Apr 1987, *Boom & Gopaul* 7698 (holotype NY; isotypes BM, BRG, PMA, UC, US).

Etymology—Named after the type locality.

Plants terrestrial. Stems creeping, stramineous, 20-34 (or more) cm long, 0.9-1.6 mm diam., not articulate, not flagelliform, not stoloniferous, 2- or 3(-4)- branched. Rhizophores axillary or occasionally with both axillary and dorsal rhizophores, restricted to basal $\frac{1}{2}$ of the stem, stout, (0.44)0.54-0.88 mm diam. Leaves dimorphic throughout, membranaceous, the upper surface green, composed of $\pm$ rounded cells with slightly sinuate walls. Lateral leaves distant, perpendicular to the stem, oblong-ovate to oblong, 3-5.3 x 1.5-2.6 mm; base subcordate to rounded, the acroscopic base strongly overlapping the stem, the basisopic base not overlapping the stem; margins hyaline to pale green, the acroscopic margin serrate, the basisopic margin entire to serrulate apically; apex rounded to obtuse, dentate at tip; both surfaces glabrous, the upper surface with conspicuous idioblasts and without stomata, the lower surface with obscure idioblasts and with stomata along midrib. Median leaves distant, ascending, ovate to broadly ovate, 2-3.1 x 1-1.7 mm; base subcordate, the inner base rounded and the outer base with a well developed auricle; margins hyaline, serrate; apex long-acuminate to short-aristate, the acumen or arista $\frac{1}{4}$ or less of the length of the lamina, 0.28-0.54 mm long, puberulent and dentate at tip; both surfaces glabrous, the upper surface with conspicuous idioblasts, without stomata. Axillary leaves similar to lateral leaves or more cordiform. Strobili terminal on branch tips and main stem apex, slightly lax and flattened, dorsiventral, 3-13 mm long. Sporophylls dimorphic, basally attached to the axis; dorsal sporophylls green, spreading, with an adaxial laminal flap extending along ca. $\frac{1}{2}$ to $\frac{1}{3}$ the sporophyll length, asymmetric or lanceolate to lance-ovate, 1.54-2.08 x 0.6-0.92 mm, with a strongly developed and denticulate keel along midrib, the base rounded, the margins slightly hyaline to hyaline-greenish, serrate, the apex acute to shortly acuminate, the upper surface green, composed of $\pm$ rounded cells, except for the portion that folds to form the flap where the cells hyaline and elongate with sinuate walls, glabrous, with conspicuous idioblasts, the stomata along midrib, the lower surface silvery to silvery green, composed of elongate cell with sinuate walls, and without idioblasts; ventral sporophylls pale green, ascending to spreading, without an adaxial flap, ovate, 1.32-1.76 x 0.6-0.9 mm, with a well developed and slightly denticulate keel along midrib, the base rounded, the margins hyaline to pale green, serrate, the apex acute to shortly acuminate, the surface made of elongate cells with sinuate walls, glabrous, with idioblasts present only on the lower surface, the stomata present only along midrib of the lower surface. Megasporangia in two ventral rows; megaspores maize yellow, with a prominent equatorial flange, the proximal face striate to reticulate with open reticulum of low ridges, the distal face reticulate with open reticulum of high ridges, 320-340 μ m diam. Microsporangia mostly absent from the two dorsal rows, or few mature near the apex on dorsal rows; microspores not studied.

HABITAT AND DISTRIBUTION: On sandy or rocky soil; 920-1080 m; known only from

Karowtipu Mountain in Guyana.

PARATYPES: GUYANA. Upper Mazaruni: Karowtipu Mountain, 5°45'N, 60°35'W, 920 m, 18 Apr 1987, *Boom & Gopaul* 7415 (BRG, MO, NY!, PMA, U, UC, VEN); 920-1080 m, 24 Apr 1987, *Boom & Gopaul* 7698 (BM, BRG, NY, PMA, UC, US).

TAXONOMIC DISCUSSION: *Selaginella karowtipuensis* resembles *S. seemannii* in its median leaves with a well developed outer auricle and acuminate apex, and typically oblong to oblong-ovate lateral leaves with subcordate to rounded base. It differs from *S. seemannii* by conspicuous (vs. without) idioblasts on the upper surface of the lateral and median leaves and sporophylls. In addition, *S. karowtipuensis* is a more robust plant with stouter rhizophores (0.54-0.88 mm diam., compared with 0.25-0.65 mm diam. for *S. seemannii*).

DISCUSSION – CONCLUSION

Of the 31 species mentioned by Valdespino (1995) within this subgenus, eight occur in the Guianas. A key, extracted from his monograph, is given below.

Key to the species of *Selaginella* P. Beauv. subg. *Heterostachys* Baker in the Guianas.

1. Acroscopic margin of the lateral leaves ciliate to ciliate-denticulate at least along basal ¼ 2
- 1'. Acroscopic margin of the lateral leaves serrate to serrulate or entire near the base or throughout 6
2. Upper surface of the lateral leaves with idioblasts *S. muscosa*
- 2'. Upper surface of the lateral leaves without idioblasts 3
3. Lateral leaves semicordiform to cordiform; median leaves cordiform to ovate-deltate *S. porelloides*
- 3'. Lateral leaves ovate, ovate-orbicular, ovate-lanceolate, lanceolate, ovate oblong, or ovate-elliptic; median leaves ovate, ovate-lanceolate, lanceolate, elliptic, elliptic-orbicular, or, if ovate-deltate, then the apex broadly acute and ciliate at tip ... 4
4. Median leaves elliptic or ovate-elliptic 5
- 4'. Median leaves ovate, ovate-lanceolate, or lanceolate *S. minima*
5. Lateral leaves with an acuminate apex; upper surface of the median leaves without idioblasts; megaspores lemon yellow *S. sobolifera*
- 5'. Lateral leaves with a broadly acute to obtuse apex; upper surface of the median leaves with conspicuous to obscure idioblasts; megaspores light to maize yellow, cream, pale yellow-orange, or pale orange-yellow *S. gynostachya*
6. Upper surface of lateral and median leaves with idioblasts, or idioblasts restricted to the upper surface of the lateral leaves only, or idioblasts present on the upper surface of the median leaves and/or on the lower surface of the lateral leaves 7
- 6'. Upper surface of the lateral and median leaves without idioblasts, or idioblasts present only on the upper surface of the median leaves 9
7. Median leaves with at least the outer base well developed into an auricle free from the stem; rhizophores 0.3-0.86 mm diam *S. karowtipuensis*
- 7'. Median leaves with the outer base asymmetric, rounded, oblique, or subcordate, if with an outer auricle then this not distinctly free from the stem; rhizophores 0.08-0.56 mm diam. 8

- 8. Apex of the lateral leaves obtuse to broadly acute; apex of the median leaves acute to shortly acuminate, the acumen 1/8 or less the length of the lamina ... *S. gynostachya*
- 8'. Apex of the lateral leaves acute to shortly acuminate; apex of the median leaves long acuminate to long-aristate, the acumen or arista 1/4 or usually more the length of the lamina *S. muscosa*
- 9. Median leaves with the outer base well developed into an auricle *S. seemannii*
- 9'. Median leaves with the outer base asymmetric, oblique or semicordate, without a well developed auricle 10
- 10. Median leaves with the apex acute, acuminate, or short-aristate, the acumen or arista 1/3 or less the length of the lamina *S. gynostachya*
- 10'. Median leaves with the apex long-aristate, the arista more than 1/3 the length of the lamina *S. flagellata*

DISTRIBUTION

When comparing the distribution of the 35 species of subgenus *Heterostachys* from Central and South America, it is noticed that Venezuela has the highest diversity with 17 species. The Guianas have fewer: four for Guyana, two for Suriname and five for French Guiana. This is unusual because French Guiana typically has fewer species than Guyana. In terms of distribution, a few species occur over widespread areas (such as *S. flagellata*), whereas others are limited to the Plateau of the Guianas (*S. gynostachya*), or simply known only from Guyana (*S. karowtipuensis*).

ACKNOWLEDGEMENTS

We would like to express our gratitude and thanks to Drs. T. Lobova, J.T. Mickel, R.C. Moran and S.A. Mori (NYBG) who have kindly provided access to the specimens and a scan, to the technical staff of the National Museum of Natural History in Paris (P) who carried out one scan, and to Dr. J.J. de Granville (CAY Herbarium, French Guiana) who has made the detailed photographs of the specimens.

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SYSTEMATICS OF *TRICHOMANES* (HYMENOPHYLLACEAE: PTERIDOPHYTA), PROGRESS AND FUTURE INTERESTS

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Key words: filmy ferns, *rbcL*, *Trichomanes*

ABSTRACT

Trichomanes L. sensu lato (s.l.), is a large group of Hymenophyllaceae to which ca. 250 species are attributed, distributed from the tropics to temperate regions around the world. Their life forms and morphology are more diversified than those of the other large filmy-fern genus *Hymenophyllum*. Phylogenetic analyses were performed based on the *rbcL* sequences of 81 *Trichomanes* taxa, covering most of the major groups within the genus, in addition to morphological, anatomical and cytological investigations, that offer a number of insights concerning evolution of the genus. Eight robustly supported clades are recognized within *Trichomanes*, while some traditional trichomanoid taxa (e.g., *Pleuromanes*) are transferred to the *Hymenophyllum* clade.

INTRODUCTION

Because of their simplified morphology among pteridophytes, filmy ferns (Hymenophyllaceae) have attracted the attention of many researchers, especially those interested in evolution and phylogeny. The family's basal placement among leptosporangiate ferns was already suggested by morphological evidence (oblique annuli of sporangia; Bower, 1926) and supported by recent molecular phylogenetic work (Pryer *et al.*, 2004). In contrast, it is difficult to reconstruct its intrafamilial lineages from its morphological characters, probably as a result of numerous parallel evolutions (Dubuisson, 1997a). Several different classification systems (e.g., Copeland, 1938; Morton, 1968; Iwatsuki, 1984) are currently in use for this family.

Dubuisson (1997b) first adopted molecular phylogeny to infer intrafamilial relationships among the Hymenophyllaceae, specifically targeting one of the two largest groups, *Trichomanes* sensu lato (s.l.), to explore the reliability of the chloroplast *rbcL* marker at the infrageneric level. The result showed better resolution than for the other group, *Hymenophyllum* s.l., in which less genetic variation was found for *rbcL* (Pryer *et al.*, 2001; Ebihara *et al.*, 2002; Hennequin *et al.*, 2003). *Trichomanes*, however, is a large genus comprising around 250 species (Iwatsuki, 1990) occurring nearly throughout the tropics and extending into the temperate zone, especially the southern latitudes. Despite the study by Dubuisson *et al.* (2003a) focusing on Neotropical *Trichomanes*, many distinctive Palaeotropical taxa remained unsampled. Recently, Ebihara *et al.* have added considerable new *rbcL* data, making a global revision of the genus possible.

COVERAGE OF SAMPLING

In total, 81 species of *Trichomanes*, approximately a third of the estimated number of extant species, were included in this study, though some traditional trichomanoid taxa (*Microtrichomanes* Copel. pro parte and *Cardiomanes reniforme* (G.Forst.) C.Presl) whose affiliations to the *Hymenophyllum* lineage have already been suggested (Pryer *et al.*, 2001; Ebihara *et al.*, 2004) were not counted as *Trichomanes*. This sampling covered all of Copeland's trichomanoid genera (*Abrodictyum* C.Presl, *Callistopteris* Copel., *Cephalomanes* C.Presl, *Crepidomanes* (C.Presl) C.Presl, *Crepidopteris* Copel. [= *Reediella* Pic.Serm.], *Davalliopsis* Bosch, *Didymoglossum* Desv., *Feea* Bory, *Gonocormus* Bosch, *Lecanium* C.Presl, *Macroglena* (C.Presl) Copel., *Microgonium* C.Presl, *Nesopteris* Copel., *Pleuromanens* (C.Presl) C.Presl, *Polyphlebium* Copel., *Selenodesmium* (Prantl) Copel., *Trichomanes* sensu stricto (s.s.) and *Vandenboschia* Copel.) and most of Morton's (1968) sections under *Trichomanes*, except for four small sections of the subgenus *Achomanes* C.Presl (sections *Odontomanes* (C.Presl) C.Chr., *Trigonophyllum* (Prantl) C.Chr., *Homoeotes* (C.Presl) C.Chr. and *Ragatelus* (C.Presl) C.Chr.).

A data matrix consisting of 1206 bp fragments of the *rbcL* sequences from 81 *Trichomanes* species as well as 12 other Hymenophyllaceae and 4 non-Hymenophyllaceae (*Polypodium glycyrrhiza* D.C.Eaton, *Matonia pectinata* R.Br., *Osmunda cinnamomea* L. and *Angiopteris evecta* Hoffm.; the last taxon was treated as an outgroup) was analyzed using the maximum-parsimony (MP) method with PAUP*4.0 software (detailed in Ebihara *et al.*).

EVOLUTIONARY RELATIONSHIP

In the resulting consensus MP tree (Ebihara *et al.*, in prep.; Figure 1), both the *Trichomanes* and *Hymenophyllum* clades are strongly supported, as in Pryer *et al.* (2001) and Ebihara *et al.* (2004). *Pleuromanens*, a group which has always been considered a member of *Trichomanes*, is indeed embedded in the *Hymenophyllum* clade and eight robustly-supported subclades (BS > 90) are recognizable in the *Trichomanes* lineage. These groupings do not match any of the existing classifications, but seem to be closely related to the plants' morphological characters, chromosome base numbers and geographical distributions.

The phylogenetic framework suggests three evolutionary scenarios for the genus: (1) Cytological data reveals that the chromosome base numbers are constant within each "clade," so the observed diversity in number may originate from aneuploid reduction from the ancestral $x = 36$. (2) Life form data indicate that four monophyletic clades (Cr, Di, Pa and Va in Fig. 1), consisting mostly of epiphytic and epipetric taxa displaying evolutionary traits that tend towards morphological and anatomical reductions, are supported with fairly high reliability (Ebihara *et al.*). Assuming that the common ancestor of *Trichomanes* was terrestrial, the occurrence of epiphytism related to evolutionary regression was apparently quite a significant event in the genus, and at least four independent evolutionary transitions led to the epiphytic habit. (3) Geographical distribution data show that four of the eight clades are subcosmopolitan; the NT clade is nearly confined to the Neotropics, while the Ca, Ce and Cr clades are confined to the Palaeotropics. Peculiar monotypic trichomanoid "genera" distributed in the southern hemisphere, which formed the basis of the Antarctic origin theory of Hymenophyllaceae (Copeland, 1938, 1939), are all placed in derivative positions in the present phylogeny.

FUTURE CHALLENGES

Usefulness of rbcL

In the case of *Trichomanes*, *rbcL* phylogeny is a useful tool for inferring the “macroevolution” or global relationships among sublineages of the genus. Indeed, eight principal clades have recently been defined as eight distinct genera (Ebihara *et al.*). Although our results show that there is some useful genetic variation for discussing relationships among species with closely-related *rbcL* sequences, further consideration is necessary before adopting chloroplast-coding markers for this purpose. If hybridization or reticulate evolution (e.g., Wagner 1954; van den Heede *et al.*, 2003; Ebihara *et al.*, 2005) occurred in a clade, the relationship between the members as reconstructed from chloroplast DNA, which is maternally inherited in ferns (Gastony & Yatskievych, 1992), would not be accurate.

For example, Copeland’s genus *Gonocormus*, ranging from Africa to the Pacific region, consists of several taxa segregated by leaf shape and proliferation (e.g., *Trichomanes saxifragoides* C.Presl, *T. minutum* Blume and *T. proliferum* Blume); however, Yoroï and Iwatsuki (1977) argued that it should be difficult to recognize such taxa morphologically and tentatively clustered them into a single species, *T. minutum* (Iwatsuki, 1984). Although much genetic variation is also found in *rbcL* sequences taken from several specimens of the *Gonocormus* species collected from various localities, there does not seem to be a clear relationship between their morphological and genetic variation (Ebihara, unpublished data). Considering the fact that diploid, triploid and tetraploid series are reported for *Gonocormus* (Braithwaite, 1969, 1975; Yoroï & Iwatsuki, 1977), reticulate evolution has probably occurred in this complex. Another example is the *Trichomanes* (*Vandenboschia*) *radicans* complex (the Va clade), which includes the American *T. radicans* Sw., the European *T. speciosum* Willd., the African *T. giganteum* Willd., the Japanese *T. orientale* C.Chr. and the Asian *T. birmanicum* Bedd. Our study, utilizing the biparently-inherited nuclear *GapCp* marker, suggests that hybridization involving at least three biological units occurred in Japan, despite an observed difference in *rbcL* of up to 1.66% (20/1206 bp; Ebihara *et al.*, 2005) among them. In addition, it is likely that both *T. speciosum* and *T. giganteum* are of hybrid origin (Ebihara, unpublished data). Considering the high incidence of allopolyploid hybridization in ferns (Soltis & Soltis, 1999), such examples may be ubiquitous rather than exceptional. These results strongly indicate the importance of biparently-inherited markers in species-level analyses. Analyses using sequences from multiple gene regions are also necessary to clarify relationships among the clades that are unresolved in the present *rbcL* phylogeny.

Importance of field observations

The evolution of life forms is one of the most interesting topics concerning Hymenophyllaceae. Their habits are usually divided into several types, such as terrestrial, epiphytic, epipetric, hemi-epiphytic and true lianas (Dubuisson *et al.*, 2003b), but in fact their ecological classification is routinely complex and requires careful and precise *in situ* observations. For example, we have never seen herbarium specimens of *T. auriculatum* with underground parts (roots). Rather, most herbarium specimens consist only of epiphytic parts, i.e., leaves with climbing rhizomes. Our field observations, however, reveal that the plant germinates on the ground and the rhizomes climb up tree trunks. Field investigations are therefore essential for evolutionary studies.

Diversification history and conservation

Extant filmy ferns exclusively prefer moist and shady environments, but the habitat where their common ancestor acquired its unique one-cell thick lamina in the Triassic or earlier (AxSmith *et al.*, 2001; Pryer *et al.*, 2004) remains unknown for certain. The fact that roughly half of the Hymenophyllaceae species are attributed to the hymenophylloids, which are mostly epiphytic on tree trunks and diversified relatively recently compared to trichomanoids (Hennequin *et al.*, 2003; Schuettpeltz & Pryer, 2006), is consistent with the trend of extant fern diversification in angiosperm forests (Schneider *et al.*, 2004). Our phylogeny also suggests that a few species in *Trichomanes* with an epiphytic habitat on trees may have recently acquired this habit.

Filmy ferns are one of the fern groups most sensitive to environmental changes, particularly decreased humidity caused by deforestation. Habitat conservation and conservation biology studies of already-endangered species (e.g., Rumsey *et al.*, 1998) are crucial for the future study of this family and to maintain its present diversity.

ACKNOWLEDGEMENTS

The authors are grateful to Dr. H. Schneider for valuable comments on the manuscript.

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**SPATIAL PATTERN OF INDIVIDUAL GENETIC SIMILARITIES
IN POPULATION OF *ASPLENIUM CETERACH*
(*ASPLENIACEAE*: *PTERIDOPHYTA*)**

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Keywords: intrapopulation structure, *Aspleniaceae*, RAPD, UPGMA

ABSTRACT

The genetic relatedness of individuals can give a population finer scale spatial pedigree structure. The relation of genetic similarity and spatial distance refers to the dispersal characters and reproductive relations existing among individuals. Our main purpose here was to obtain information on the genetic spatial pattern before a more profound spatial autocorrelation analysis of *Asplenium ceterach* individuals. Three physically isolated subpopulation patches of the tetraploid *A. ceterach* subs. *ceterach* were identified on the southern rocky faces of the St. György Hill in Hungary. The genetic properties were scored and cluster analysis, UPGMA, was carried out in three steps using progressively larger samples: 42, 85 and 320 individuals were chosen. Cluster analysis revealed a minimum of 70% genetic similarities among individuals indicating intensive gene flow between subpopulations, but there was also detectable correspondence between individual genetic similarities and spatial position.

INTRODUCTION

The homosporous, autotetraploid, xerophytic rock fern, *Asplenium ceterach* L. subsp. *ceterach* is one of three cytotypes of the species *Asplenium ceterach* (Reichstein 1981, Vida 1965, 1973). *Asplenium ceterach* and related taxa are circumscribed by special morphological characters within *Asplenium*. The taxonomic treatment of these ferns have been the subject of debate: some authors have recognised *Ceterach* as a separate genus within *Aspleniaceae* (Reichstein, 1981), but others consider it as a clade or subgenus within the genus *Asplenium* (Pintér *et al.* 2002, Viane *et al.* 1993).

The study of geographical distribution and patterns of genetic diversity within *Asplenium ceterach* has revealed its postglacial colonisation (Trewick *et al.* 2002). The European pteridophyte flora has an ancient origin with the Mediterranean areas having a major role as refugia during the glaciation periods (Vogel *et al.* 1999). Several experimental studies demonstrate that autopolyploids are derived from diploids (Manton, 1950; Reichstein, 1981; Vida, 1973). Although the ancestral (diploid) and derived (polyploid) taxa have the same substrate preference, they have distinct geographical distributions. During the glaciation periods the Balkans and Italy acted as refugia for *Asplenium ceterach*. The diploids are mostly restricted to the Mediterranean basin (Pintér *et al.* 2002.), whereas the tetraploids occupy areas further north in Europe. The wide occurrence of the tetraploid *Asplenium ceterach* subsp. *ceterach* across

Europe can be explained by its better dispersal capacity owing to single spore colonisation in this taxon (Vogel *et al.* 1999). Analysing the haplotype patterns of European *Asplenium ceterach* has elucidated recurrent polyploid formation and colonisation from different population sources throughout Europe (Trewick *et al.* 2002). The tetraploid subspecies, *Asplenium ceterach* subsp. *ceterach*, is common and widespread in Europe but in Hungary, diploid *Asplenium ceterach* subsp. *bivalens* predominates in natural habitats and the occurrence of tetraploid populations has been reported only from the St. György Hill and the Buda Hills parts of the Transdanubian Mountains (Vida 1973, Vida 1965). On the bases of chloroplast DNA analyses two different haplotype sequences were identified in Hungary ('red' and 'green' according to Trewick *et al.* 2002.). The haplotype sequence 'red' is found both in diploids and tetraploids, whereas the 'green' haplotype is found only in tetraploids. Diploid populations with the 'red' haplotype are described mainly from the Balkans, while tetraploid populations of 'red' and 'green' haplotypes are rather frequent in South-Western Europe (Trewick *et al.* 2002). The tetraploid *Asplenium ceterach* populations on the St. György Hill studied by us have the 'green' haplotype (Morgan-Richards, Vogel unpublished). While the volcanic soil of the hill has been utilized for vine-growing, the steep rocky slopes are natural habitats. These dry rocks may have colonized after the last glaciation. Here, six physically isolated subpopulation patches of *A. ceterach* subsp. *ceterach* were identified on the southern rocky faces of the hill (Figure 1).

The genetic relatedness of individuals can reveal a fine-scale spatial pedigree structure in a population (Vekemans & Hardy 2004). The relationship of genetic similarity and spatial distance reflects the dispersal characters and reproductive relationships among individuals (Hardy 2003). The aim of this study was to examine the genetic constitution of this particular tetraploid population in the context of the past colonisation events before a more profound spatial autocorrelation analysis. The analysis of the population's genetic constitution and its spatial structure as revealed by molecular markers can provide information about microevolutionary processes occurring when the individuals are linked by bonds of mating and parenthood (Hardy & Vekemans 2002).

Genetic data derived from allozyme studies indicate that tetraploid *Asplenium ceterach* shows high genetic variation between populations and low within population variation, unlike diploids (Vogel *et al.* 1999). Genetic differences between diploid and tetraploid populations result from differences in their breeding systems. The high degree of population differentiation in the tetraploid taxon is caused by low level of outcrossing. (Vogel, pers. com.). There was no evidence of vegetative propagation among our cultivated individuals, nor are there any reported cases in the literature.

MATERIAL AND METHODS

In this study we present detailed information about the genetic relatedness of individuals in a tetraploid population. For measuring spatial distances the exact position of individuals was mapped (see below). This provides a 3D coordinate system for the finer scale spatial analysis that follows. Use of a large number of RAPD products gives a powerful tool for documenting offspring-parent relationships and dispersal distances (Levitan and Grosberg 1993, Hadrys 1992) especially at lower taxonomic levels (Rodrigues *et al.* 2002.).

Sampling strategy

Individuals of *Asplenium ceterach* were collected from the southern rocky faces of St. György Hill near Lake Balaton in Hungary. Here, six physically isolated subpopulation patches were identified from where we collected 440 leaf samples (1 leaf per individual). To obtain information on the genetic spatial pattern we surveyed three out of six patches with progressively larger samples. The sample size was increased by adding new individuals to the previously selected ones. These samples included 42, 85 and 320 individuals.

Constructing the spatial position map of the individuals

The task was to score the genetic properties of individuals and to give their exact position in space in order to construct the 3-dimensional population genetic structure before embarking on the fine scale analysis. For describing individual plants' location on the hill, GPS localized reference points were used. The WGS84 GPS-based coordinates were converted to the Hungarian National Grid (EOV) (Timár *et al.* 2002, Molnár & Timár 2002, Timár & Molnár 2002) (Figure 2). Reference coordinates were the starting points of a grid, and before calculating the coordinates of each individual in 3D space. This coordinate system can be matched to a geographical map. The positional data formed the basis for the future analysis of the genealogical connections.



Figure 1. St. György Hill and the subpopulation patches of *A. ceterach subsp. ceterach* (1a, 1b, 2) on the southern rocky faces of the hill.

Genetic studies

Genetic studies on the reliability of RAPD markers prove that this method is as suitable for determining genetic identity as the AFLP techniques (Albert *et al.* 2003). Bias of polymorphic band selection is the weak point of this method. Many polymorphic bands can be generated by only a few RAPD primers (Stewart & Porter 1995), but a few are reliable. The applied bands have to be selected carefully. In our case, the decreased amount of primers might influence precise indication. However, polymorphic bands obtained from several primers give a more reliable result.

For total plant DNA isolation we used DNeasy Plant Mini Kit (Qiagen). The extraction protocol was slightly modified for pteridophyte material. A dry mass of leaves, 100mg of tissue was ground in liquid nitrogen the presence of Polyclar AT. A volume of 600µl extraction buffer and 6-8µl Rnase (100mg/ml), and 16-17µl β-mercaptoethanol were added. This suspension was homogenized and incubated at 65°C for more than 1 hour with frequent and careful mixing. After adding 195-200µl precipitation buffer, the mixing was repeated. This was followed by 10 minutes incubation on ice and centrifugation at 12,000 rpm for 5-8 mins. The supernatant was removed and applied to the QIAshredder column, combined in a 2ml eppendorf and was centrifuged at 8000 rpm for 2 mins. Taking 1.5:1 proportion of the AP3/E and the liquid that came down the column and it was mixed carefully again. This suspension was centrifuged through the DNeasy mini column at 8000rpm for 1 min. Next a washing buffer was added, followed by centrifugation at 8000rpm for 1min. This process was repeated and an additional centrifugation step was applied for 3 mins.

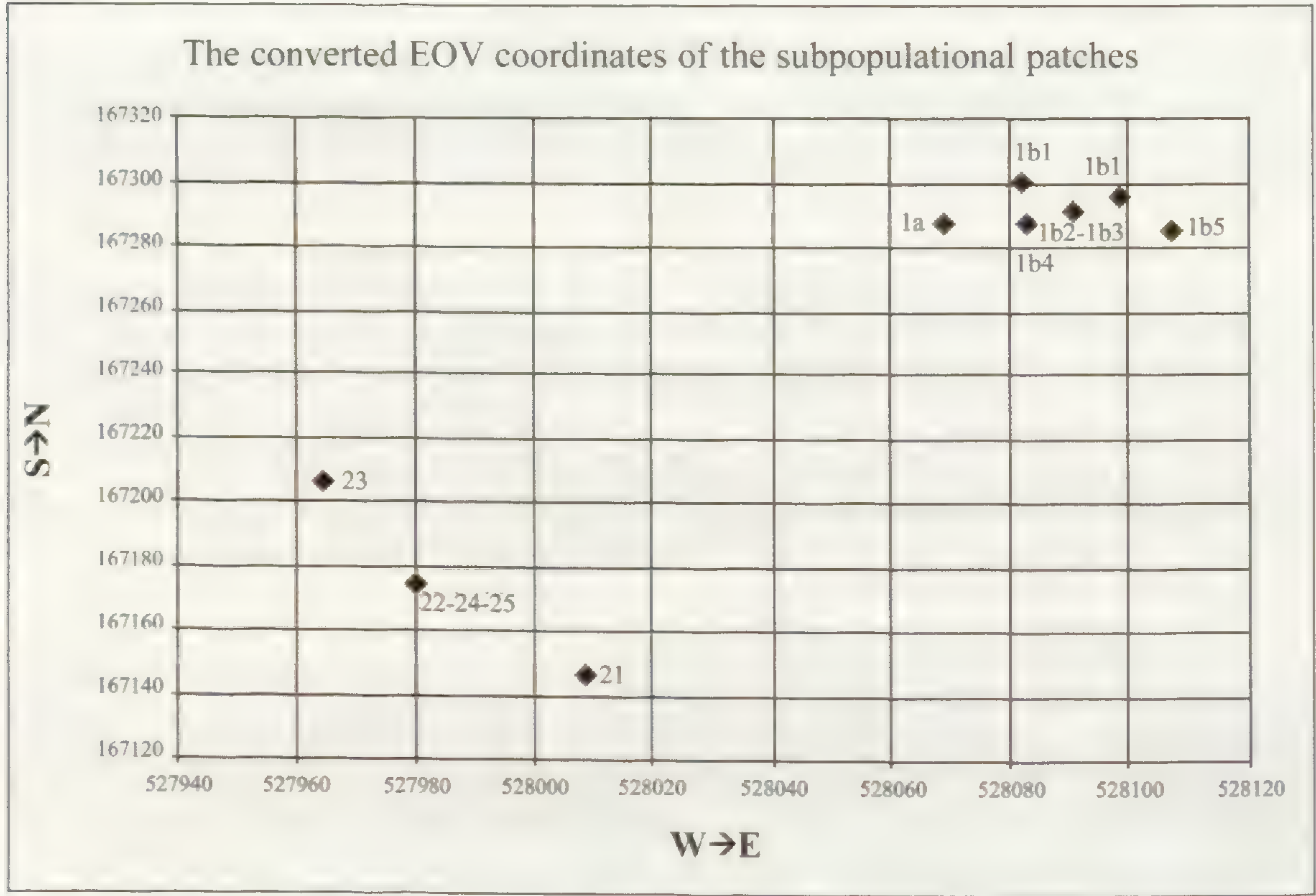


Figure 2. To describe the location of individual plants on the hill, GPS localized reference points were used. The WGS84 GPS-based coordinates were converted to the Hungarian National Grid (EOV) (Timár *et al.* 2002, Molnár & Timár 2002, Timár & Molnár 2002).

Finally the DNS was eluted twice in 100µl and 50µl. The DNA samples were then either stored at +4°C for immediate use or stored at -20°C.

For the DNA concentration quantification a self-made DNA standard was used. Its concentration was measured by lambda 35 spectrophotometer and UV winlab program. The DNA samples were diluted to 1ng/ µl.

RAPD procedure

For genetic identification the multilocus genotype (phenotype) of RAPD polymorphic loci was used. The RAPD method uses a large set of oligonucleotide primers annealing with various parts of the genome. RAPD markers produce good Mendelian characters, typically, but not always dominant (Hillis *et al.* 1996). For the generation of genetic data 60 oligonucleotide primers 10 basepairs long were screened. The best 8 were: OPA-7, OPA-19, OPB-18, OPK-9, OPK-19, OPP-4, OPP-5, OPP-14. These primers generated strong, clear and repeatable polymorphic bands.

For PCR reaction we applied 5µl (1ng/ µl) DNA template. The PCR reactions were carried out in a PerkinElmer thermocycler. The amplification cycles ran: 60 sec at 94°C, two cycle of 30 sec at 94°C, 30 sec at 41°C, 120 sec at 72°C, 20 cycles of 30 sec at 94°C, 15 sec at 39°C, 15 sec at 45°C, 90 sec at 72°C, 18 cycles of 30 sec at 94°C, 15 sec at 39°C, 15 sec at 45°C, 120 sec at 72°C and the final cycle 72°C 5min (Schneller 1998.).

A 4-5 hour long electrophoresis at 180-200V was used for the separation of the fragments.

Data Analysis

The polymorphic bands of RAPD markers were scored 1 for presence and 0 for absence. We applied simple matching coefficient for the dissimilarity matrix (Sokal & Michener 1958). $SM = a+d / a+b+c+d$; (a, d: the numbers of the binaric variable coincide, b, c: the numbers of the binaric variable do not coincide.)

For cluster analysis the **UPGMA** (unweighted pair group method with arithmetic mean) method was used. The complete multivariate analysis and its presentation were managed with the **NTSYS-pc program Version 2.** (Rohlf 2000, Podani 1997). The results were illustrated with dendrograms reflecting the genotypic (phenotypic) similarity between the objects.

The programmes SAHN module offers two options to deal with ties. The FIND option constructed each alternative clustering. As there were several ties in the dissimilarity matrix we had maximum 50 output trees. It was possible to compute their cophenetic correlation to check how well the different tied trees represent the original distance matrix (Rogrigues *et al.* 2002.). First the COPH module computed a cophenetic (ultrametric) value matrix from each dendrogram. These matrices are used to test the goodness of fit of clustering analysis by using MXCOMP module (Rohlf and Sokal 1981). The module evaluates the cophenetic correlation (product moment correlation: r) between the cophenetic value and the original distance matrices. We selected trees with the highest evaluated correlation coefficient from among the tied tries. The normalized Mantel test was also performed. The Mantel test statistic Z is frequently used to measure the correspondence between matrices (Mantel 1967, Podani 1997).

RESULTS

Our main purpose was to obtain information on the genetic spatial pattern before a more profound spatial autocorrelation analysis of 440 positioned *Asplenium ceterach* individuals. Based on the scored genetic properties and their spatial position the individuals were clustered in three steps using progressively larger samples: 42, 85 and 320 individuals were chosen. The progressive increase of the size of the samples may indicate the sensitivity of the spatial pattern analysis to the sample size.

The multivariate analysis of RAPD data revealed a high level of genetic relatedness among either individuals or subpopulations. In all samples the assessment of the minimum genetic similarity between individuals was approximately 70%, and at least half of the plants showed 80-90% genetic similarity. The high level of genetic relatedness between individuals either from the same or different subpopulations suggested a high level of gene flow between the different subpopulations. Since individuals show close genetic relatedness the UPGMA produced several alternative clusters. The cophenetic correlations did not detect important difference in goodness of fit among the dendrograms. Similar moderate correlation coefficient values were computed for each alternative dendrogram. The matrix correlations (= normalized Mantel stat Z) for different dendrograms were between $r=0.62-0.65$ for 42 individuals, $r=0.71-0.73$ for 85 individuals and $r=0.618-0.625$ for 320 individuals. The Mantel test gave p (probability random $Z < \text{observed } Z$) = 1 for 1000 permutations for each tested dendrogram. We selected one tree with the highest evaluated cophenetic correlation coefficient (product moment correlation: r) (Rohlf and Sokal 1981) (Figures 3 & 4).

The first two sample sets (42, 85) showed a similar range of SM values for the clusters (Figures 3, 4). The SM index varied from 70% up to 100%. In half of the sampled individuals a similarity of more than 90% was detected. The genetic similarity of the common individuals in the first two sample set was practically the same. Among the first 42 individuals two homogenous subclusters were recognizable with 80-83% similarity level ('subpopulations 1a2' and '2'). In the sample of 85 individuals another homogenous 'subpopulation 2' cluster appeared with approximately 80% similarity. The individuals of these two 'subpopulation 1a2' and '2' also displayed their cohesiveness (Figure 5). The rest of the dendrogram did not show any distinguishable cluster.

The first two samples with 42 and 85 individuals were relatively small compared with the total number of individuals. The doubling of the sample size did not alter the intrapopulational spatial genetic pattern. The next step was the analysis of 320 individuals. The dendrogram constructed with 320 individuals showed not only quantitative but also qualitative change in the picture of the spatial pattern. Increasing the sample size gave a more detailed spatial pedigree structure along the dendrogram. Subpopulations appeared as slightly separated subclusters of the tree. The minimum similarity value for these characterized subclusters was around 77-80% (The minimum genetic similarity between any two individuals is 70%). These distinguishable substructures may demonstrate a significant number of non-random events in the reproductive processes and propagation. This tree represents pedigree structure of these ferns, and it differs from random. Large number of individuals appear to be outside their characteristic subpopulational clusters scattered along the dendrogram.

The cluster analysis revealed minimum 70% genetic similarities among individuals. The spatial genetic pattern indicates intensive gene flow between subpopulations, but

also there was detectable correspondence between genetic similarities and spatial position. This dual spatial pattern can be derived from the two step process of pteridophyte reproductive biology and haploid-diploid life cycle, as the spores and gametes have different dispersal capacities.

DISCUSSION

Asplenium ceterach is a homosporous fern with hermaphroditic gametophytes. The spatial pattern of the genetically related individuals within the population is influenced by both reproductive biology and dispersal characteristics. According to Klekowski's terminology two different kinds of mating types might produce different levels of heterozygosity: intragametophytic selfing, yielding completely homozygotic sporophytes (with the theoretical possibility of homeologous heterozygosity), and intergametophytic mating between two sib or non-sib gametophytes resulting in varying degree of heterozygosity (Klekowski 1979). The tetraploid *Asplenium ceterach* is reported as a highly successful in-breeder inferred from allozyme data (Trewick *et al.* 2002.).

The alternating haploid-diploid life-cycle is an important characteristic of ferns (Page 2002). Spores and gametes have different dispersal capacity. The distance between two gametophytes accomplishing mating is very much limited in space (Suter 2000). However, the distance over which spores travel is comparatively unlimited. This kind of haplotype transport can reorganize the population structure. Yet some authors propose that 95% of the spore dispersal is within 1-10m from the parent plant (Vogel *et al.* 1999.) The question is how the mating system and spore dispersal affect

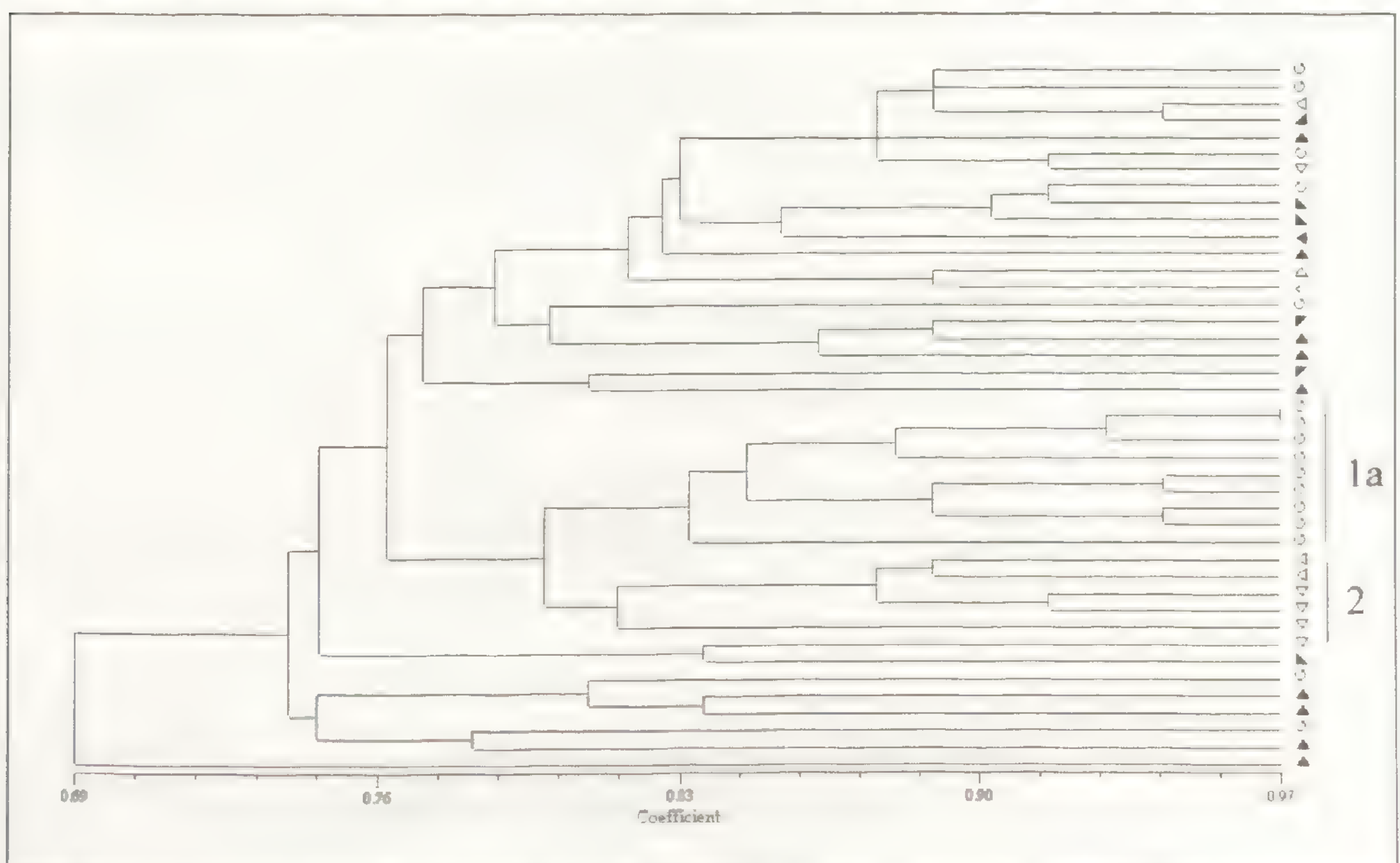


Figure 3. Cluster analysis of 42 fern individuals. The individuals in the dendrogram are labelled according to their subpopulational (1a, 1b, 2) and intra-subpopulational (1a1, 1a2, 1b1, 1b2, 1b3, 1b4, 1b5, 21, 22, 23, 24, 25) positions:

Subpopulations and their symbols:

1a1:	1a2:	1b1:	1b2:	1b3:	1b4:	1b5:	21:	22:	23:	24:	25:
↺	↻	◀	◀	◀	◀	▲	◁	△	▽	▷	◄

intrapopulation genetic pattern. The genetic similarity of neighbouring individuals may help elucidate the nature of breeding system. Cluster analysis of the RAPD multilocus phenotype suggests high levels of gene flow between these distant subpopulations and, likewise, a correspondence between individual genetic similarities and spatial position. Since the subpopulations are further than 10m apart, this suggests that spore dispersal should exceed 10 m. But cluster analysis is not reliably informative to dissect the influence of spore travel from the influence of mating type in the determination of individuals genetic relatedness. This can be carried out by further, more profound spatial autocorrelation analysis.

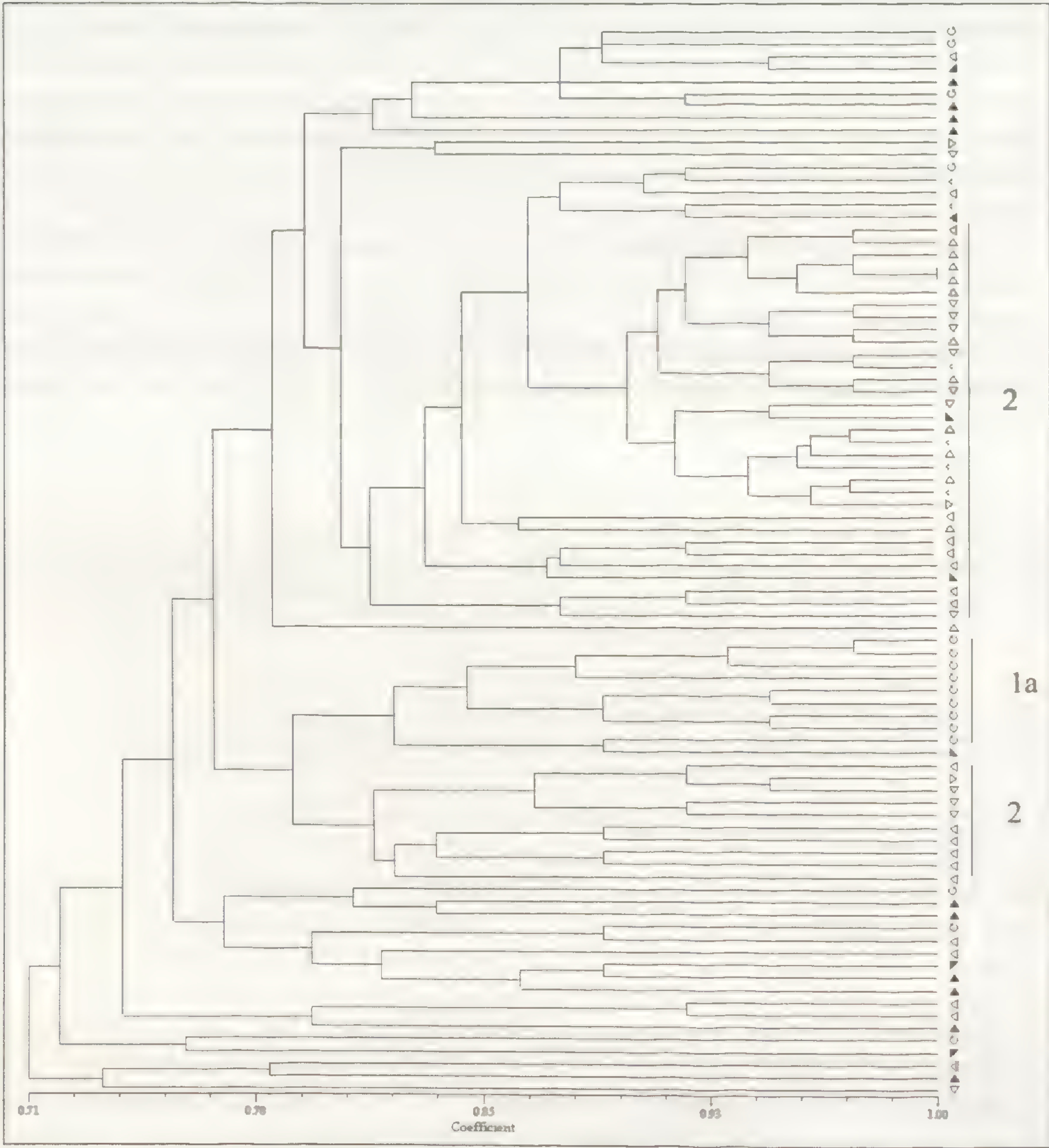


Figure. 4. Cluster analysis of 85 fern individuals. The individuals in the dendrogram are labelled according to their subpopulational (1a, 1b, 2) and intra-subpopulational (1a1, 1a2, 1b1, 1b2, 1b3, 1b4, 1b5, 21, 22, 23, 24, 25) positions:
Subpopulations and their symbols:
1a1: 1a2: 1b1: 1b2: 1b3: 1b4: 1b5: 21: 22: 23: 24: 25:
⤿ ⤿ ◀ ▶ ▲ ▴ ◁ ▷ ▽ ▹ ◀

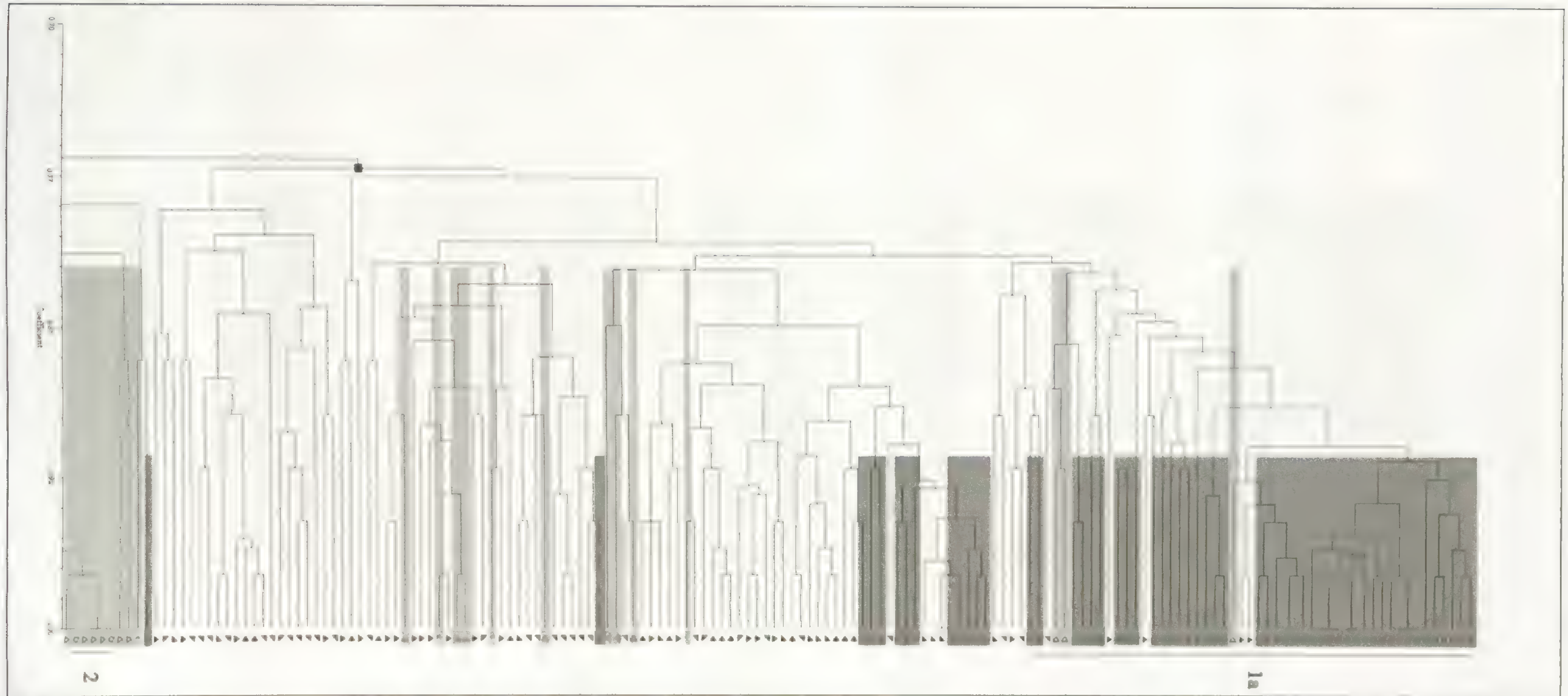


Figure. 5a. Cluster analysis of 320 fern individuals. The individuals in the dendrogram are labelled according to their subpopulational (1a, 1b, 2) and intra-subpopulational (1a1, 1a2, 1b1, 1b2, 1b3, 1b4, 1b5, 21, 22, 23, 24, 25) positions:

Subpopulations and their symbols:

1a1:	1a2:	1b1:	1b2:	1b3:	1b4:	1b5:	21:	22:	23:	24:	25:
G	C	▲	▼	▴	▾	▶	△	▷	◁	▽	◀

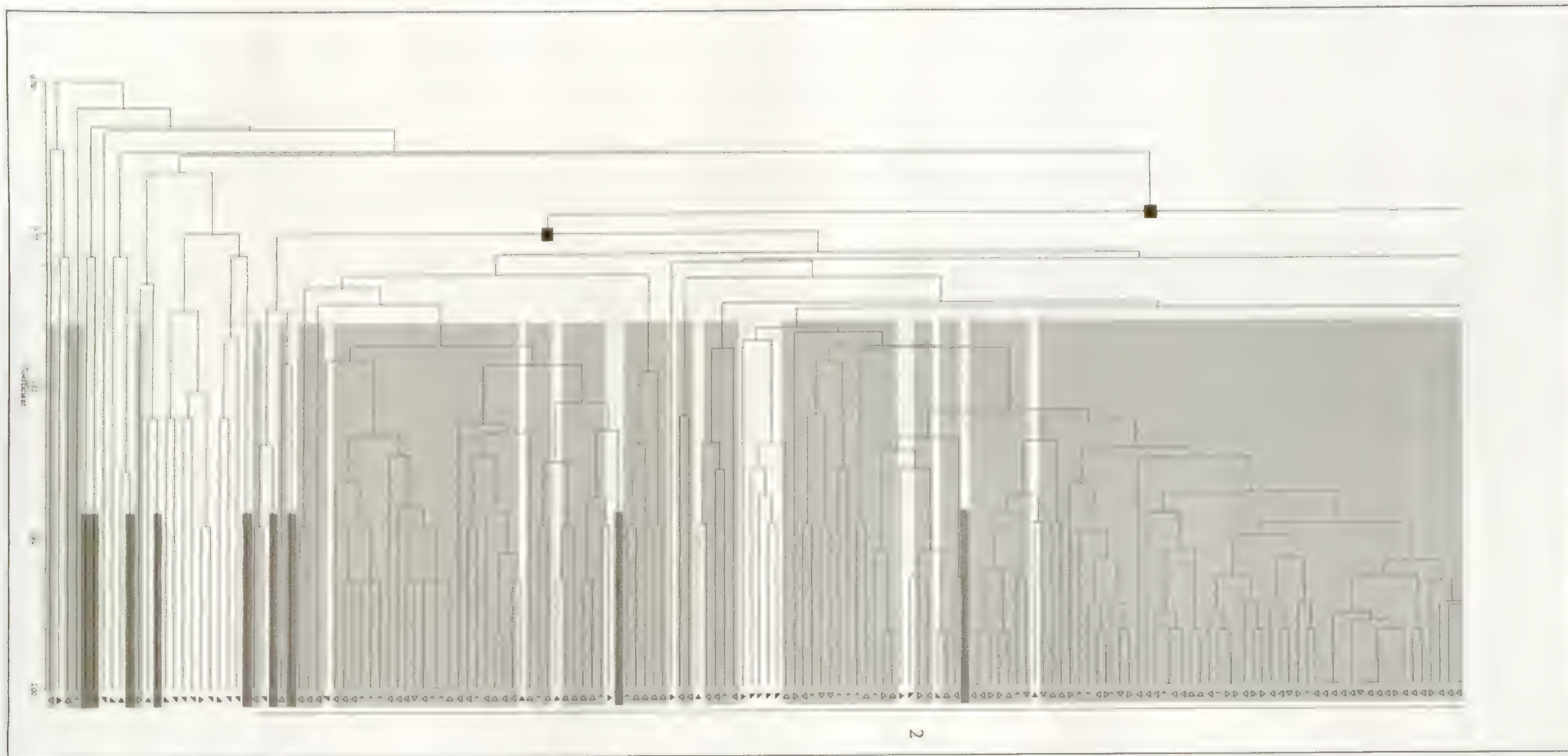


Figure. 5b. Cluster analysis of 320 fern individuals. The individuals in the dendrogram are labelled according to their subpopulational (1a, 1b, 2) and intra-subpopulational (1a1, 1a2, 1b1, 1b2, 1b3, 1b4, 1b5, 21, 22, 23, 24, 25) positions:

Subpopulations and their symbols:

1a1:	1a2:	1b1:	1b2:	1b3:	1b4:	1b5:	21:	22:	23:	24:	25:
⬢	⬢	▲	▼	▲	▼	▶	△	▷	◁	▽	⬆

ACKNOWLEDGEMENTS

I would like to express my thanks to Maria Takács and Maria Tuschek for their kind help, and many thanks for Gábor Timár and Bálint Halpern for their help in the GPS work.

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ASSESSING THE CONSERVATION STATUS OF PTERIDOPHYTES, A CHALLENGE FOR THE GLOBAL STRATEGY FOR PLANT CONSERVATION

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Key words: conservation status, Global Strategy for Plant Conservation,
Pteridophytes, Red Data Lists.

ABSTRACT

The Global Strategy for Plant Conservation (GSPC) sets out a series of activities with targets intended to halt the decline in plant biodiversity by 2010. This article examines the current state of knowledge concerning the conservation status of ferns in relation to Target 2 of the GSPC. The change in criteria used by the World Conservation Union (IUCN) for assessing conservation status has led to data generated prior to 1997 being marginalised. The latest information, using the revised criteria and published in 2003, refers to a smaller number of pteridophyte species, with only ten species being common to both the 1997 and 2003 assessments. There is an urgent need to capture the knowledge that pteridologists and other specialists undoubtedly have, relating to the conservation status of ferns and fern allies, and to incorporate this into Red Data Lists to provide firmer foundations for the GSPC.

INTRODUCTION

The biodiversity crisis affecting our world is undoubtedly a real problem (Pimm *et al.*, 1995; Heywood & Watson, 1995; Gomez-Pompa, 2004) and, like global warming with which it is intimately connected (Thomas *et al.*, 2004), it poses great challenges to the future of humanity (Wilson, 2002). The biodiversity crisis might, however, be much more amenable to solution than global climate change if appropriate practical conservation policies are implemented at the local level around the world. The Global Strategy for Plant Conservation (GSPC) adopted in 2002 under the Convention on Biological Diversity sets out measurable targets as milestones towards halting the loss of plant biodiversity by 2010 (Anon, 2003). At present, however, the rate of progress on global conservation programmes is severely constrained by the lack of investment in biodiversity science (see for example, Blackmore, 2002; House of Lords, 2002; Wheeler *et al.*, 2004).

The global status and conservation of pteridophytes has been the focus of a recent conference, the proceedings of which provide an excellent overview (Dyer *et al.* (eds), 2002) and there are many published case studies for particular taxa. The purpose of this contribution is to review the current state of knowledge about pteridophytes in relation to selected targets of the GSPC. The point we wish to emphasise is that whilst the extant pteridophytes are less species rich than angiosperms and might therefore seem an easier group to conserve, much of the basic information needed to do this effectively is lacking. In particular we highlight the paucity of information concerning the conservation status of pteridophytes.

CONSERVATION STATUS OF PTERIDOPHYTES

The first three targets of the GSPC are all concerned with understanding and documenting plant diversity and concern the underpinning knowledge needed for conservation efforts to be demonstrably effective. Whilst most plant taxonomists are aware of the extent to which the initial exploration and documentation of biodiversity is incomplete, it continues to surprise many people, including policy makers, that many new species are discovered each year and how incomplete the documentation of life on earth is. Perhaps the most widely authoritative estimate, derived from the global biodiversity assessment (Heywood & Watson, 1995), is that 1.7 million species have so far been described out of an estimated total of 13 million. However, current estimates are approximate at best and the question of how many flowering plant species there are, for example, has seen much recent discussion. For pteridophytes, Roos (1996) estimated that between 10,500 and 11,300 species have been described from a total of between 12,000 and 15,000. In other words somewhere between 6% to as many as 30% of pteridophytes species have not yet been discovered. This has an important bearing on Target 1 of the GSPC: the preparation of "a widely accessible list of all plants, as a step towards a complete world flora." The importance of the target derives from the widely acknowledge fact that one cannot be certain of conserving species without even knowing that they exist, although one important aspect of protected areas, especially in biodiversity hotspots is that, hopefully, they will harbour a good proportion of the unknown taxa. The technical rationale accompanying Target 1 makes it clear that a working list rather than a definitive list of species is the objective for 2010. Any such list can, by definition, only be a list of the known species and thus for pteridophytes our current state of knowledge would permit a list that contains somewhere between 70% and 94% of the estimated world total. It is encouraging to note that good progress has already been made towards the achievement of Target 1. The international plant names index (www.ipni.org) provides a highly accessible database of names and associated bibliographic information and now incorporates the data from the published volumes of *Index Filicum* (Johns, 1996) on the names of ferns and fern-allies. Web-based resources are not only the most accessible means of delivering such information but, in many ways, also the most appropriate, given that they can be updated much more readily than printed literature. The world of ferns website (<http://homepages.caverock.net.nz/~bj/fern/>) is another highly accessible source of information on names and synonymy in pteridophytes.

A much less favourable situation applies to Target 2: "a preliminary assessment of the conservation status of all known plant species, at national, regional and international levels". As the technical rationale for Target 2 makes clear, the conservation status has so far been assessed for over 60,000 plant species, of which 34,000 are classified as globally threatened with extinction (Anon, 2003) and, given the vast amount of working still to be done, the intention is to undertake a preliminary assessment for the "data-deficient" species by 2010. The scale of this challenge emphasises just how little information we have to hand on the conservation status of plants in general. The situation for pteridophytes will be explored in more detail.

Information derived from the IUCN website (www.iucnredlist.org) in June 2004 indicated that there are 13,025 known species of pteridophytes. Of these, conservation status has only been determined for 180 of these species and as a result 111 species were

considered to be threatened in 2003. Although, on this information, only 1% of the total number of species of ferns and fern allies are known to be threatened, with two species being extinct, 62% of those that have actually been evaluated are considered to be threatened. More complete information on the status of 186 species of pteridophytes in 2003 from the IUCN web site is summarised in Table 1. Clearly we are in a state of relative ignorance about the true scale of the threats to pteridophytes species. If 62% of all pteridophytes were threatened, this would constitute some 8,075 species. There is, of course, no sound scientific basis for making such a projection.

It is interesting to compare this most recent summary of knowledge with earlier information available on the web. The web site of the World Conservation Monitoring Centre (www.unep-wcmc.org) presents information from the earlier assessment contained in Walter & Gillett (1997). According to this source, a more comprehensive survey of the conservation status of pteridophytes had already been previously undertaken (Table 2). In 1997, using the IUCN's earlier system for evaluating threats, 768 species of pteridophytes had been assessed (compared to just 111 under the criteria in use in 2003), of which nine were extinct in the wild and a further 13 were suspected of being extinct. The 1997 Red Data List (Walter & Gillett, 1997) did not list species that were of indeterminate status or were not threatened.

Although good reasons were advanced for changing the criteria and categories for threatened plants between the 1997 and the 2003 assessments, the fact remains that hard-earned information recorded prior to 1997 was marginalised as a result. We estimate that preparation of the 1997 Red List required the equivalent of about 250 person years of work. Furthermore, very little re-assessment of previously studied species, to bring the pre-1997 data into the new system, has been undertaken. Only ten species out of the 768 species assessed as threatened in 1997 have been reassessed in the 2003 list (Table 3). In general it is easy to see, from the list of those species that occur in both the 1997 and the 2003 list, that reassessment is either not taking place or is restricted to assessments of particular groups of endemic species, such as the

Table 1. Data on pteridophytes from the 2003 Red Data List. EX = extinct, EW = extinct in the wild, CR = critically endangered, EN = endangered, VU = vulnerable, NT = near threatened, LC = least concern, DD = data deficient. Species listed as NT, LC or DD are not considered to be threatened and therefore the total number of threatened pteridophytes detailed in Table 1 is 111, comprising those listed as CR, EN, or VU.

	EX	EW	CR	EN	VU	NT	LC	DD	Total
LYCOPSIDA	0	0	1	2	8	1	1	0	13
SELAGINELLOSIDA	0	0	0	0	1	1	0	0	2
ISOETOPSIDA	0	0	0	0	1	0	0	0	1
POLYPODIOPSIDA	2	0	26	22	50	12	7	45	164
Totals	2	0	27	24	60	14	14	45	186

pteridophytes of Ascension Island.

As a consequence of the extremely low rate of re-assessment and because of the change in criteria it is almost impossible to extract evidence of trends in the conservation status of pteridophytes, or other plants. We consider that this poses one of the greatest challenges to the GSPC. Although we strongly support efforts to move ahead with data gathering under the new system we are concerned that this is not happening rapidly enough. The reasons for this are difficult to determine but we suspect that for many species, especially for endemics, more information is known, informally by specialists on particular taxa or localities, than has been recorded. We therefore urge pteridologists, and other botanists, to enter such information as they do have into the IUCN system.

The importance of a better state of knowledge on the conservation status of ferns impacts on other targets within the GSPC. For example, Target 8 aims to have 60% of the world's threatened species conserved *in situ* and 10% of them to be included in species recovery programmes. On the basis of the Red List data 2003, this would require only 66 threatened species of fern to be conserved *in situ*, with 6 being the subject of recovery programmes. That has probably already been achieved but this is hardly a satisfying observation in that it reflects ignorance rather than progress.

CONCLUSIONS

Current syntheses of the documented taxonomic diversity of pteridophytes provide a sound basis for the achievement of a preliminary checklist of the world's ferns and fern allies in the context of Target 1 of the GSPC. This is not to deny the need for significant further revisionary work to define taxonomic concepts, particularly for large and complex genera. Syntheses of information on the conservation status of known pteridophytes are, in contrast, woefully inadequate. This results in part from changes of

Table 2. Summary of the status of pteridophytes recorded in the 1997 Red List (Walter & Gillett, 1997). Abbreviations: Ex = extinct, Ex/E = possibly extinct, E = endangered, R = rare, V = vulnerable, I = indeterminate. Total threatened species is the number of species assessed as Ex/E, E, V, R or I (in other words, it excludes extinct species). % threatened is the total threatened species as a percentage of the number of known species.

	Ex	Ex/E	E	V	R	I	Total Threatened Species	Number of known species	% Threatened
LYCOPODIOPSIDA	1	0	3	6	11	3	23	519	4.4
SELAGINELLOPSIDA	1	0	3	5	9	6	23	713	3.2
ISOETOPSIDA	2	0	11	4	22	2	39	79	49.7
POLYPODIOPSIDA	5	13	85	97	356	133	683	9053	7.5
Totals	9	13	102	112	398	144	768	10,364	7.4

criteria used for evaluating conservation status but even more fundamentally from the low proportion of known species for which assessments of status have been recorded. There is now an urgent need to harness the expertise of people who know about pteridophytes and their status and to complete the recording of conservation status if we are to understand the conservation needs of ferns and fern allies properly. This case study focuses on pteridophytes but it is likely that a similar situation applies to plants in general. We end by urging pteridologists and others to familiarise themselves with the procedures for assessing conservation status (see: www.iucn.org/themes/ssc/redlists/RLcats2001booklet.html).

Table 3. Summary information on the ten species of pteridophytes that feature in both the 1997 and the 2003 Red Lists with an assessment of whether their status in the wild appears to have improved or worsened and their geographical distribution.

	1997 Red List	2003 Red List	Change in Status	Distribution
<i>Anogramma ascensionis</i>	Ex/E	Ex	Unchanged	Ascension
<i>Asplenium ascensionis</i>	R	NT	Improved	Ascension
<i>Ctenitis squamigera</i>	E	CR	Unchanged	Hawaii
<i>Cyathea bipinnata</i>	R	VU	Worse	Ecuador
<i>Cyathea heliophila</i>	R	EN	Worse	Ecuador
<i>Dryopteris ascensionis</i>	Ex/E	Ex	Unchanged	Ascension
<i>Elaphoglossum pellucidum</i>	R	CR	Worse	Hawaii
<i>Marattia purpurascens</i>	R	NT	Improved	Ascension
<i>Pteris adscensionis</i>	E	CR	Unchanged	Ascension
<i>Xiphopteris ascensionense</i>	R	NT	Improved	Ascension

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BOOK REVIEW

ENCYCLOPEDIA OF GARDEN FERNS. Olsen, S. 2007. Hardback, 444pp., 692 colour photographs, 2 line drawings. ISBN 13: 978-0-88192-819-8. Timber Press, Portland. US\$59.95; £40.00. 11" x 8" (28cm x 22cm).

This is a highly informative and enjoyable book that most fern enthusiasts will wish to own. It is intended primarily for fern collectors and growers, amateur and commercial (see the review in *Pteridologist*, 2007), but there is much to interest fern biologists. Its considerable size and weight, and large number of colour photos, suggest a 'coffee table book' but these usually receive superficial attention and Sue Olsen's book will be regularly consulted for detailed information on identification, cultivation and, in many cases, natural habitats of nearly 1000 species. Despite its all embracing title, there is a strong North American emphasis and the book is similar in scope to John Mickel's *Ferns for American Gardens* (second edition 2003, first edition 1994, reviewed *American Fern Journal* 84 (3), p.104), but contains almost twice as many species. There is an overlap also with Rickard's book *The Plantfinder's Guide to Garden Ferns* (2000, published in UK by David & Charles, Newton Abbot, reviewed *Fern Gazette* 16(3), pp. 146, 167) but Rickard writes from a British perspective and gives more attention to the horticultural cultivars popular during the British fern craze. A strong case can be made for acquiring all three books because of their complementary strengths.

Olsen's Encyclopedia contains 5 chapters, 8 Appendices, a Glossary, a Reference List and an Index. The 'ferns' of the title are taken to include not only horsetails, as we are now required to do, but also *Psilotum* and the lycophytes *Diphasiatrum*, *Huperzia*, *Lycopodiella*, *Lycopodium* and *Selaginella*. *Isoetes* and *Tmesipteris* are not, however, discussed. An initial preamble includes acknowledgement of the owners of gardens where she has taken photographs. Amongst these are several past and present BPS members including ex-Presidents Martin Rickard and Alastair Wardlaw. Then four short chapters deal with, in turn, "Ferns through the ages", "Cultivating ferns", with guidance on topics from garden habitats to companion planting, "Propagating ferns", with descriptions of propagation techniques together with an outline of the typical life cycle, and "Fern structure and basic diagnostics" with a brief introduction to the main characters, including frond morphology, used in fern recognition.

Chapter 5, "Ferns from around the world", is the heart of the book and, with 320 pages, the major part of it. Nearly 1000 fern species are arranged in alphabetical order of genera; about 700 are accompanied by colour photographs. For most species the entry begins with the Latin name, the common name, the translation of the Latin specific epithet, the height, whether it is "evergreen" or "deciduous", and the plant hardiness zone(s) to which it is adapted. Then follows a concise but informative description of diagnostic features, an indication of its natural geographic range and habitat, and guidance on cultivation, together with additional helpful comments when appropriate. Some less frequently cultivated species are represented by a brief entry under "Shorter Notes".

In the Appendices are a Plant Hardiness Zone map for the USA, a comparable map for Europe (unfortunately with a different colour coding), a list of ferns that have been awarded an RHS Garden Merit Award, and a list of ferns designated "Great Plants" by the Elisabeth C. Miller Botanical Garden, Seattle. Appendix 4 consists of "Top twenty ferns" lists provided by 19 enthusiasts from USA, UK and Germany and representing

hardiness zones from 4 to 11. Appendices 5 to 8 provide recommendations for ferns for special situations, a brief introduction to fern societies (are the BPS, AFS and the Hardy Fern Foundation really the only fern societies?), a list of gardens noted for ferns, and a list of commercial growers. The book finishes with a glossary of about 120 essential terms including “discombobulated: the feeling an uninitiated fern amateur might have when encountering technical terms”, a somewhat idiosyncratic list of books and papers referred to in the text, and an index of Latin and common plant names.

The list of featured species is impressive but nevertheless represents less than 10% of the world flora. The criteria for selecting the chosen species are not explicitly stated but most of the ferns mentioned are temperate species hardy at temperatures below freezing during winter (Hardiness Zone 9 or below), thereby eliminating most ferns, which are tropical. Many of those chosen are familiar garden subjects but I cannot believe that all of them were listed because they are commercially available. Ferns from all over the world are represented, but not every fern in cultivation is included. Even for Britain, the list is not quite complete. For example, *Cystopteris dickieana* is recognised in the text as a distinct species and shown in a photograph (p.168) but there is no descriptive entry for it even though this attractive dwarf fern is easy to grow, and plants propagated from spores collected at the type locality in a Scottish seaside cave have been widely distributed, at least among British fern enthusiasts. I suspect that the list simply represents those species which can be grown somewhere in the USA or are otherwise known to the author. Nevertheless, it will be possible to identify most garden ferns using the descriptions and illustrations provided.

With such an eclectic list of ferns, it is important to provide guidance to the readers about which ferns will grow in their area. Guidance on suitable climatic conditions is given by means of Plant Hardiness Zones, based on the “average annual minimum temperature” and thus a guide to how cold it can get in winter. This guidance is widely used in the USA but it is less familiar in the British Isles, perhaps because it is not sufficiently discriminatory. Most of Britain away from the coast, from Wick to Winchester, is in Zone 8. In continental Europe, Zone 8 extends from Stavanger to Salamanca. In eastern USA, Zone 8 extends from southern North Carolina to northern Florida. Clearly, few if any ferns will grow equally well throughout Zone 8 because of other environmental factors. Hardiness Zones are, at best, broad indicators of which ferns are worth trying.

In the introductory chapter on propagation (p.74), there is a brief account of hybrid formation, but the treatment of fertile allopolyploids is inconsistent and potentially confusing to the uninitiated. For example, there is no indication that research has revealed that *Polystichum aculeatum* is the allotetraploid hybrid of two ancestral species, *P. setiferum* and *P. lonchitis*, formed by ancient cross-fertilisation followed by chromosome doubling. However, tetraploid *Dryopteris filix-mas*, with a similar type of history thought to involve *D. caucasica* (or something similar) and *D. oreades*, is stated to be “*Dryopteris caucasica* x *D. oreades*” and within the description is mention of the fact that it is a fertile hybrid. *Woodsia alpina*, another allotetraploid species, is defined as “*Woodsia glabella* x *W. ilvensis*” and this time, its status as “a fertile hybrid” is emphasised in the first sentence of the description. Some readers may not realise that all three species have arisen in a similar way, and that indeed many fern species are fertile polyploids derived from two or more diploid ancestors. In the absence of any mention of ploidy or chromosome number in the species entries, they might also find it difficult to distinguish these fertile polyploids of ancient hybrid origin from recent,

sterile, hybrids with no chromosome doubling. There is also an inconsistent treatment of these recent hybrids. For example, *Dryopteris x australis* (p.214) and *D. x boottii* (p.216) are correctly presented as recent sterile natural hybrids but a similar hybrid, *D. x complexa*, is described as fertile without explaining that the apparent fertility is due to inherited apomixis (apogamy plus diplospory) rather than to the compatibility of the parental genomes. To compound the confusion, *Dryopteris x remota*, a recent rare hybrid of *D. affinis* and *D. expansa*, is listed as "*D. remota*", implying a species, but then described as a "fertile hybrid", spore production being again due to inherited apomixis. A consistent treatment of hybrids, and some explanation of the role of hybridisation and polyploidy in species formation and evolution in ferns, perhaps in Chapter 1 "Ferns through the Ages", would have been helpful.

Whilst acknowledging that fern gardening is all about the decorative sporophytes, in a book called an Encyclopedia I would like to have seen a little more about the vital gametophyte generation. There is a very brief mention in the outline of the fern life cycle (p.69) and in the account of propagation (p.72), and on p.151 the author comments that *Blechnum spicant* is "the only species where I have seen prothalli in nature". The ecology of gametophytes in natural habitats is largely unknown and cannot therefore inform the choice of conditions for propagation from spores. However an indication of the diversity of form of gametophytes, with photographs, would have been welcome. I would also expect a mention of the perennial gametophytes that can be cultivated, but *Anogramma* is not included, even as a sporophyte, and under *Trichomanes* (p.389) the statement that filmy ferns are "sometimes only seen in a permanent state without sporophytes" will not be easily understood by readers who are not familiar with examples of independent gametophytes.

There are commendably few mistakes or typographical errors, though on p.69, "one pound [2kg] of spores" leaves the reader wondering whether *Cyathea medularis* produces 454gm or 2000gm of spores each year.

To the grower, I would suggest that you use the horticultural information in this book as a useful guide but don't be inhibited by indications that a species might not be successful in your conditions; you might be pleasantly surprised. Not knowing that *Ceterach officinarum* is "wretchedly difficult" (p.49), I wedged my only plant between small rocks at the foot of a south-east facing slope and, some ten years later and with no further assistance other than some lime chippings, it is still growing, slowly but steadily. Less than 2m away I have three plants of the calcifuge *Cryptogramma crispa* of similar age and also slowly increasing even though this species is said by Olsen to be "almost impossible to cultivate" (p.180). Although these successes are more due to a lucky combination of garden conditions than to horticultural prowess, it does demonstrate that whatever the advice, it is always worth trying a fern you wish to grow, preferably with some attempt to imitate its natural habitat, and in several different conditions of aspect, shade and moisture.

To the fern biologist, I would say don't dismiss this book as solely for gardeners. It is a goldmine of interesting information on the ecology and growth requirements of a wide selection of species.

A.F. Dyer

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STEVENSON, D.W. & LOCONTE, H. 1996. Ordinal and familial relationships of pteridophyte genera. In: CAMUS, J.M., GIBBY, M. & JOHNS, R.J. (Eds) Pteridology in perspective, pp. 435-467. Royal Botanic Gardens, Kew.

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THE FERN GAZETTE

VOLUME 18 PART 2

2007

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ISSN 0308-0838